PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 6084 KEL/CTG:SCJ	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 00/01026**	International filing date *(day/month/year)* 30 August 2000	Priority Date *(day/month/year)* 30 August 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] A61K 31/275 A61P 1/18

Applicant

1 KELLY,E.,Lyndell

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **4** sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
13 March 2001	23 August 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer TAMARA NIZNIK Telephone No. (02) 6283 2422

International application No.
PCT/AU 00/01026

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages , as originally filed,
pages , filed with the demand,
pages **4,** received on with the letter of **13 March 2001.**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **25-27,** received on with the letter of **13 March 2001.**

☒ the drawings, pages **1/21-21/21,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of .

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of .

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

International application No.
PCT/AU 00/01026

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-16	YES
	Claims	NO
Inventive step (IS)	Claims 1-16	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-16	YES
	Claims	NO

Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purpose of this report :

D1: Pancreas, vol 6, no 2, pp 168-174, 1991
D2 : Food and Chemical Toxicology. vol 26, no 2, pp 137-147, 1988
D3 : Fundamental and Applied Toxicology, vol 14, pp 144-159, 1990
D4 : Biochemical and Biophysical Research Communications, vol 246, pp 476-483, 1998

NOVELTY (N)

Claims 1-16 meet the criteria set forth in PCT Article 32(2)-(4) for novelty. The prior art published before the priority date does not disclose a method of providing selective, substantially total, non-regenerative apoptosis of pancreatic acinar cells by administering a single dose, subcutaneously or intra-arterially of a composition of cyanohydroxybutene.

Document D1-D3 discloses only oral administration of cyanohydroxybutene for acinar cell death.
Document D4 discloses a single dose of cyanohydroxybutene by intravenous administration.
Therefore the subject matter of these claims is new and the claim meets the requirements of Article 33 (2) PCT with the regard to requirement for novelty.

INVENTIVE STEP (IS)

Claims 1-16 meet the criteria set out in PCT Article 33 (3) with regard to the requirements of Inventive Step because the prior art does not obviously suggest to a person skilled in the art that subcutaneous injection of cyanohydroxybutene (CHB) would cause apoptosis of the substantially entire population of acinar cells, without the side effect caused by high doses of CHB with oral or intravenous administration.

Therefore the subject matter of these claims is not obvious and meets the requirements of Article 33 (3) PCT with regard to the requirement for inventive step.

International application No.
PCT/AU 00/01026

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of :Box 1

Rule 67 lists the subject matter which under Article 34(4)(a)(i) an international preliminary examination is not required to be carried out. At item (iv) it specifies methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods, as such matter. However the agreement between WIPO and Australia further qualifies this by excepting from exclusion any subject matter which is examined under national grant procedures. Claims 1-16 have nonetheless been considered because the identified subject matter does not contravene Australian law.

14

PATENT COOPERATION TREATY

PCT

REC'D 13 SEP 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 6084 KEL/CTG:SCJ	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 00/01026**	International filing date *(day/month/year)* 30 August 2000	Priority Date *(day/month/year)* 30 August 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] A61K 31/275 A61P 1/18

Applicant

1. KELLY,E.,Lyndell

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **4** sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
13 March 2001	23 August 2001
Name and mailing address of the IPEA/AU	Authorized Officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	TAMARA NIZNIK Telephone No. (02) 6283 2422

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages , as originally filed,
pages , filed with the demand,
pages **4,** received on with the letter of **13 March 2001.**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **25-27,** received on with the letter of **13 March 2001.**

☒ the drawings, pages **1/21-21/21,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of .

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of .

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-16	YES
	Claims	NO
Inventive step (IS)	Claims 1-16	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-16	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purpose of this report :

D1: Pancreas, vol 6, no 2, pp 168-174, 1991
D2 : Food and Chemical Toxicology. vol 26, no 2, pp 137-147, 1988
D3 : Fundamental and Applied Toxicology, vol 14, pp 144-159, 1990
D4 : Biochemical and Biophysical Research Communications, vol 246, pp 476-483, 1998

NOVELTY (N)

Claims 1-16 meet the criteria set forth in PCT Article 32(2)-(4) for novelty. The prior art published before the priority date does not disclose a method of providing selective, substantially total, non-regenerative apoptosis of pancreatic acinar cells by administering a single dose, subcutaneously or intra-arterially of a composition of cyanohydroxybutene.

Document D1-D3 discloses only oral administration of cyanohydroxybutene for acinar cell death.
Document D4 discloses a single dose of cyanohydroxybutene by intravenous administration.
Therefore the subject matter of these claims is new and the claim meets the requirements of Article 33 (2) PCT with the regard to requirement for novelty.

INVENTIVE STEP (IS)

Claims 1-16 meet the criteria set out in PCT Article 33 (3) with regard to the requirements of Inventive Step because the prior art does not obviously suggest to a person skilled in the art that subcutaneous injection of cyanohydroxybutene (CHB) would cause apoptosis of the substantially entire population of acinar cells, without the side effect caused by high doses of CHB with oral or intravenous administration.

Therefore the subject matter of these claims is not obvious and meets the requirements of Article 33 (3) PCT with

International application No.
[redacted]T/AU 00/01026

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of :Box 1

Rule 67 lists the subject matter which under Article 34(4)(a)(i) an international preliminary examination is not required to be carried out. At item (iv) it specifies methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods, as such matter. However the agreement between WIPO and Australia further qualifies this by excepting from exclusion any subject matter which is examined under national grant procedures. Claims 1-16 have nonetheless been considered because the identified subject matter does not contravene Australian law.

Summary of the Invention

The present invention arises from the surprising discovery that subcutaneous injection of CHB produces an unusual and unsuspected result of acinar cell apoptosis of normal acinar cells and acinar cell carcinoma. It was found that subcutaneous injection of CHB at an appropriate sub-lethal dosage caused apoptosis of the substantially entire population of acinar cells. The pancreatic lesion was unusual in that there was observed a marked early edema with limited inflammatory infiltration, rapid synchronous onset of acinar cell apoptosis and advanced atrophy with only a severely limited regenerative response. The application of this discovery to treat acinar cell carcinoma has led to the development of the current invention.

The present invention in one aspect broadly resides in a method of providing selective, substantially total, non-regenerative apoptosis of pancreatic acinar cells comprising a single-dose, subcutaneous or intra-arterial administration of a composition of cyanohydroxybutene and a pharmacologically acceptable aqueous carrier

An amount within the therapeutic window is the amount below a lethal dose that has the desired therapeutic effect. Preferably, the therapeutic window is selected whereby CHB serum levels are maintained at a level and for a period sufficient to cause apoptosis of substantially the whole acinar cell population, whilst remaining below the threshold serum levels that cause undesirable levels of liver damage.

The patient may be selected whereby the acinar cells being treated include acinar carcinoma cells. The acinar carcinoma cells may be presenting as localized to the pancreas or as metastases.

The administration is preferably subcutaneously, intramuscularly or intra-arterial catheter direct to the pancreas. These modes of administration appear to

CLAIMS:

1. A method of providing selective, substantially total, non-regenerative apoptosis of pancreatic acinar cells comprising a single-dose, subcutaneous or intra-arterial administration of a composition of cyanohydroxybutene and a pharmacologically acceptable aqueous carrier.

2. A method according to claim 1, wherein said therapeutic window is selected to minimise liver damage in said patient.

3. A method according to claim 1 or 2, wherein said administration is subcutaneous.

4. A method according to any one of claims 1 and 3, wherein said cyanohydroxybutene is administered at a dosage within the range of 140-160 mg CHB/kg of body weight.

5. A method according to any one of claims 1 to 4, wherein said patient is selected on the basis of said pancreatic acinar cells including acinar carcimoma cells.

6. A method for treating pancreatic disease including administering to a patient a single-dose, subcutaneous or intra-arterial, therapeutically effective amount of cyanohydroxybutene wherein said amount is sufficient to cause selective, substantially total, substantially non-regenerative apoptosis of acinar cells in the patient.

7. A method of treating a subject having a pancreatic carcinoma involving acinar cells and including the steps of:

preparing a cyanohydroxybutene (CHB) formulation; and

administering subcutaneous or intra-arterial single dose of a CHB formulation to said subject in an amount sufficient to cause selective, substantially total, substantially non-regenerative apoptosis of malignant acinar cells in a patient.

8. A method as claimed in claim 7 wherein the CHB dose is within a range of 125-160 mg CHB/kg of body weight.

9. A method as claimed in claim 8 wherein the CHB dose is within the range of 140-160 mg CHB/kg of body weight.

10. A method as claimed in claim 7 wherein the carcinoma involves either acinar cell carcinoma or pancreatic carcinoma containing a mixed population of cells including acinar cells.

11. A method as claimed in claim 7 wherein said CHB molecule is conjugated to a ligand which is selected to to bind to an acinar cell surface receptor.

12. A method according to any one of claims 7 to 11, wherein said dose is selected whereby liver damage in the subject is minimised.

13. A method of treating acute or chronic pancreatitis including the steps of:

preparing a cyanohydroxybutene (CHB) formulation; and

administering a subcutaneous or intra-arterial single dose of a CHB formulation to said subject in an amount sufficient to cause selective, substantially total, substantially non-regenerative apoptosis of malignant acinar cells in a patient.

14. A method of treating acute or chronic pancreatitis as claimed in claim 13 wherein the CHB dose is within a range of 125-160 mg CHB/kg of body weight.

15. A method of treating acute or chronic pancreatitis as claimed in claim 13 or 14 wherein the CHB formulation is administered by subcutaneous injection.

16. A method according to any one of claims 13 to 15, wherein said dose is selected whereby liver damage in the subject is minimised

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 6084kel	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 00/01026	International filing date *(day/month/year)* 30 August 2000	(Earliest) Priority Date *(day/month/year)* 30 August 1999

Applicant

1. KELLY, E., Lyndell .

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

[ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 00/01026

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[7]: A61K 31/275, A61P 1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT } (acinar and carcinoma) or (pancreatitis or acinar) or (pancreat and carcinoma) and cyano () hydroxy() butene or CBH
Medline and CAS} keywords as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pancreas, Vol 6, No. 2, pp 168-174, 1991 Maher, M et al "The Acute Pancreatotoxic Effects of the Plant Nitrile 1-Cyano-2-hydroxy-3-butene." see esp p 173	1-19
X	Food and Chemical Toxicology. Vol 26, No 2, pp 137-147, 1998 Wallig, M et al. "Selective Pancreatotoxicity in the Rat Induced by the Naturally Occuring Plant Nitrile 1-cyano-2-hydroxy-3-butene.", see abstract and discussion p 140	1-19
X	Fundamental and Applied Toxicology. Vol 14, pp 144-159, 1990 Wallig, M et al "Enhancement of Pancreatic and Hepatic Glutathione Levels in Rats during Cyanohydroxy butene intoxication". See pp 144-145	1-19

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2000	-6 NOV 2000

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No.: (02) 6285 3929	TAMARA NIZNIK Telephone No.: (02) 6283 2422

C (Continuation). DOCU[redacted]CONSIDERED TO BE RELEVANT[redacted]

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biochemical and Biophysical Research Communications. Vol. 246, pp 476-483, 1998. Bhatia Madhav et al "Induction of Apoptosis in Pancreatic Acinar Cells Reduces the Severity of Acute Pancreatitis" see abstract and p 476, p480-p483.	1-19

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HUGHES, E., John, L.
Davies Collison Cave
Level 3
303 Coronation Drive
Milton, QLD 4064
AUSTRALIE

Date of mailing (day/month/year)
18 février 2002 (18.02.02)

Applicant's or agent's file reference
6084kel

IMPORTANT NOTIFICATION

International application No.
PCT/AU00/01026

International filing date (day/month/year)
30 août 2000 (30.08.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

PIZZEYS PATENT AND TRADE MARK
ATTORNEYS
Level 11, Telstra House
167 Eagle Street
Brisbane, Queensland 4001
Australia

State of Nationality:
State of Residence:
Telephone No.: (07) 3221 9955
Facsimile No.: (07) 3221 8077
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

HUGHES, E., John, L.
Davies Collison Cave
Level 3
303 Coronation Drive
Milton, QLD 4064
Australia

State of Nationality:
State of Residence:
Telephone No.: 61 7 3368 2255
Facsimile No.: 61 7 3368 2262
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[X] other: PIZZEYS PATENT AND TRADE MARK

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Anne KARKACHI

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004668249

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 May 2001 (15.05.01)	
International application No. PCT/AU00/01026	**Applicant's or agent's file reference** 6084kel
International filing date (day/month/year) 30 August 2000 (30.08.00)	**Priority date** (day/month/year) 30 August 1999 (30.08.99)
Applicant KELLY, E., Lyndell	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 March 2001 (13.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

COPY

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference / 6084kel	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 00/01026	International filing date *(day/month/year)* 30 August 2000	(Earliest) Priority Date *(day/month/year)* 30 August 1999

Applicant

1. KELLY, E., Lyndell .

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the title,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 00/01026

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[7]: A61K 31/275, A61P 1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT } (acinar and carcinoma) or (pancreatitis or acinar) or (pancreat and carcinoma) and cyano () hydroxy() butene or CBH
Medline and CAS} keywords as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pancreas, Vol 6, No. 2, pp 168-174, 1991 Maher, M et al "The Acute Pancreatotoxic Effects of the Plant Nitrile 1-Cyano-2-hydroxy-3-butene." see esp p 173	1-19
X	Food and Chemical Toxicology. Vol 26, No 2, pp 137-147, 1998 Wallig, M et al. "Selective Pancreatotoxicity in the Rat Induced by the Naturally Occuring Plant Nitrile 1-cyano-2-hydroxy-3-butene.", see abstract and discussion p 140	1-19
X	Fundamental and Applied Toxicology. Vol 14, pp 144-159, 1990 Wallig, M et al "Enhancement of Pancreatic and Hepatic Glutathione Levels in Rats during Cyanohydroxy butene intoxication". See pp 144-145	1-19

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2000	-6 NOV 2000
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No.: (02) 6285 3929	TAMARA NIZNIK Telephone No.: (02) 6283 2422

International application No.
PCT/AU 00/01026

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biochemical and Biophysical Research Communications. Vol. 246, pp 476-483, 1998. Bhatia Madhav et al "Induction of Apoptosis in Pancreatic Acinar Cells Reduces the Severity of Acute Pancreatitis" see abstract and p 476, p480-p483.	1-19

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/15690 A1

(51) International Patent Classification[7]: A61K 31/275, A61P 1/18

(21) International Application Number: PCT/AU00/01026

(22) International Filing Date: 30 August 2000 (30.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2536 30 August 1999 (30.08.1999) AU

(71) Applicant and
(72) Inventor: KELLY, E., Lyndell [AU/AU]; 29 Langley Avenue, Wilston, Queensland 4051 (AU).

(74) Agent: PIZZEYS PATENT AND TRADE MARK ATTORNEYS; Level 11, Telstra House, 167 Eagle Street, Brisbane, Queensland 4001 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/15690 A1

(54) Title: TREATMENT OF PANCREATIC DISEASE

(57) Abstract: The present invention relates to the administration of cyanohydroxybutene (CHB) to eliminate acinar cells in a subject. Subcutaneous injection of CHB at a sub-lethal dosage caused apoptosis of the substantially entire population of acinar cells. The pancreatic lesion has marked early edema with limited inflammatory infiltration, rapid synchronous onset of acinar cell apoptosis and advanced atrophy with a severely limited regenerative response. There is further provided methods of treatment of acinar cell carcinoma and pancreatitis.

TREATMENT OF PANCREATIC DISEASE

Field of Invention

The present invention relates to treatment of pancreatic diseases of humans and animals. This invention has particular but not exclusive application for treatment of acinar cell carcinoma, mixed cell (including acinar cell) pancreatic carcinoma, acute and chronic pancreatitis.

Prior Art

The pancreas is a secretory gland comprising approximately 80% of acinar cells, 1% to 2% of islet cells in clusters, and 10% to 15% of single layered cuboidal ductal cells interlaced with blood vessels, lymphatics, nerves, and collagenous stroma (Evans et al. Cancer of the Pancreas. In Cancer: Principles and Practice of Oncology, 5th edition, DeVita et al. (Eds), Lippincott-Raven, New York).

Despite the large population of acinar cells, acinar cell carcinoma only accounts for 1% - 3% of pancreatic carcinomas. In addition only 5% - 10% of pancreatic carcinomas comprise mixed cell populations including acinar cells (Nonomura et al., 1992 Ultrastructural Pathology 16:317-329; Cubilla and Fitzgerald 1975 Cancer Research 35:2234-2248). Pancreatic mixed cell carcinomas and acinar cell carcinomas have been reported to be aggressive diseases with a high fatality rate (Klimstra et al., 1992, Am. J. Surg. Pathol. 16(9):815-837; Adis Editors. 1997, The Oncology Review 2-4). Surgical resection is the recommended treatment for pancreatic carcinomas. However even with reductions in operative mortality

little from its initial description in 1935 with the current 5 year survival rate being between 2% and 5% (Adis Editors. 1997, The Oncology Review 2-4).

Another disease of the pancreas is acute pancreatitis which appears to have variable severity. Acute pancreatitis appears to arise when the pancreatic duct is obstructed by a gallstone or tumour, or when toxins to the pancreas such as ethanol are ingested. Enzyme production continues causing digestion of the pancreas with often fatal results. Recurrent bouts of acute pancreatitis (or chronic pancreatitis) often result in scarring and deformation of the ductal system thereby causing localised obstruction and thus perpetuating pain, disability and digestive deficiency.

The events which regulate the severity of acute pancreatitis are unknown. Several studies, however, have shown that mild pancreatitis was found to be associated with extensive apoptotic acinar cell death while severe pancreatitis was noted to involve extensive acinar cell necrosis but very little acinar cell apoptosis (Kaiser et al. 1995, Am. J. Physiol. 269:C1295-C1304: Gukovskaya et al. 1996 Gastroenterology 110:875-884).

Administration of cyanohydroxybutene (CHB) to a subject appears to affect *inter alia* the pancreas. CHB is a glycosinolate breakdown product found in cruciferous vegetables, raw canola, and many stock feeds. It was observed that CHB administered at a daily dose of 200 mg/kg to rats by gavage for four days caused acinar cell apoptosis, inflammation, and exocrine pancreatic atrophy (Wallig et al. 1988, Fd Chem Toxic 26:137-147).

Histological and ultrastructural evaluations have been conducted on rats at different time periods after administration by gavage of 200 mg CHB/kg body weight in corn oil. These investigations revealed that as early as 4 hours after CHB administration, the pancreas exhibited abnormal pathology including mild to

moderate supranuclear vacuolation of acinar cells. After 24 hours of CHB administration, the rats exhibited acinar cell apoptosis with cytoplasmic basophilia, lack of zymogen, diffuse vacuolation, clumping of chromatin, and nuclear pyknosis or karyorrhexis (Wallig and Jeffery 1990, Fund. Appl. Toxicol. 14:144-159).

Synthetic CHB being racemic mixture of the R- and S- enantiomers administered by gavage in olive oil at doses of 25-200 mg/kg body weight causes similar effects in the pancreas of rats as naturally occurring CHB. It was observed that pancreatic edema and acinar cell vacuolation and depletion of zymogen granules occurred within hours of administration (Maher et al. 1991, Pancreas 6:168-175).

A single dose of 50 mg CHB/kg was administered intravenously to rats and found to form apoptotic bodies in the pancreas whereas a single dose of 100mg CHB/kg was found to cause severe pancretotoxicity with necrosis (Wallig et al. 1992 Fundamental Applied Toxicology 19:598-606).

In a further study the relationship between acinar cell apoptosis and the severity of pancreatitis was investigated by administering a single intravenous dose of CHB (70mg/kg) in corn oil to mice and inducing pancreatitis at varying times after CHB administration. They found that the severity of pancreatitis is reduced when the disease is induced during the period in which apoptosis is most extensive. However induction of pancreatitis either before or after the peak period of apoptosis results in pancreatic injury which is similar to that noted in animals not exposed to CHB (Bhatia et al.1998, Biochem. Biophys. Res. Commun. 246:476-483). It appears that the acinar cells may regenerate after treatment with CHB.

Summary of the Invention

The present invention arises from the surprising discovery that subcutaneous injection of CHB produces an unusual and unsuspected result of acinar cell apoptosis of normal acinar cells and acinar cell carcinoma. It was found that subcutaneous injection of CHB at an appropriate sub-lethal dosage caused apoptosis of the substantially entire population of acinar cells. The pancreatic lesion was unusual in that there was observed a marked early edema with limited inflammatory infiltration, rapid synchronous onset of acinar cell apoptosis and advanced atrophy with only a severely limited regenerative response. The application of this discovery to treat acinar cell carcinoma has led to the development of the current invention.

The present invention in one aspect broadly resides in a method of eliminating acinar cells in a patient by administration of cyanohydroxybutene in a therapeutic window selected to provide substantially non-regenerative apoptosis of said acinar cells.

An amount within the therapeutic window is the amount below a lethal dose that has the desired therapeutic effect. Preferably, the therapeutic window is selected whereby CHB serum levels are maintained at a level and for a period sufficient to cause apoptosis of substantially the whole acinar cell population, whilst remaining below the threshold serum levels that cause undesirable levels of liver damage.

The patient may be selected whereby the acinar cells being treated include acinar carcinoma cells. The acinar carcinoma cells may be presenting as localized to the pancreas or as metastases.

The administration is preferably subcutaneously, intramuscularly or intra-arterial catheter direct to the pancreas. These modes of administration appear to

provide CHB in the blood at a more even rate to form a concentration plateau and substantially reduce the height of the concentration peak of CHB. Oral and intravenous administration appears to produce concentration CHB peaks. Oral and intravenous administration of high doses of CHB causes substantial damage to the liver.

In a further aspect, this invention resides broadly in a method for treating pancreatic disease including administering to a patient a therapeutically effective amount of cyanohydroxybutene to cause substantially non-regenerative apoptosis of acinar cells in the patient.

The invention in another aspect resides broadly in a method of treating pancreatic carcinoma having acinar cells including

preparing a cyanohydroxybutene (CHB) formulation; and

administering one or more sub-lethal doses of the CHB formulation to a subject with acinar cell carcinoma, wherein the treatment causes substantially non-regenerative apoptosis of malignant acinar cells in a patient.

The CHB formulation is preferably a CHB solution wherein CHB is substantially dissolved in water. The CHB may be a natural or synthetically derived CHB.

The CHB dose is preferably calculated within a range of 5-300mg CHB/kg of body weight. Preferably the CHB dose is within the range of 125-160 mg CHB/kg of body weight. More preferably the CHB dose is approximately 150 mg CHB/ kg of body weight. The dosage may vary between subjects. Subjects include animals and people of different sizes and weight.

Preferably only one dose is administered. However a second or subsequent dose may be administered but preferably after a period of time such as a week or when the glutathione levels have returned to approximately normal levels.

Administration is preferably by means where the CHB is absorbed comparatively slowly and thus substantially avoids the development of CHB serum concentration peaks that cause undesirable levels of damage to the liver. Preferably the CHB dose is administered by subcutaneous injection, intramuscularly or intra-arterial catheter direct to the pancreas. In an alternative form the CHB dose is administered so that it is delivered directly to the acinar cells. In this form the CHB molecule may be conjugated to a ligand molecule which is able to bind to an acinar cell surface receptor thereby delivering CHB to the acinar cell.

Acinar cell carcinoma includes carcinomas of only acinar cells or of mixed cell populations with a portion being acinar cells.

The above method of treating acinar cells may be applied to the treatment of acute and chronic pancreatitis. Thus in another aspect the present invention broadly resides in a method for treating acute or chronic pancreatitis including

preparing a cyanohydroxybutene (CHB) formulation; and

administering one or more sub-lethal doses of the CHB formulation to a subject with acute or chronic pancreatitis wherein the treatment includes an amount of CHB that causes apoptosis of substantially all acinar cells and substantially no regeneration of acinar cells.

The description of the features of the method for treating acinar cell carcinoma apply also to the above method where applicable.

Brief description of the Drawings

In order that the invention may be more readily understood reference will now be made to the accompanying drawings which illustrate the experimental results and a preferred embodiment of the invention and wherein:

Figure 1 shows body weights of animals as a percentage of starting weight after a single subcutaneous injection of saline or CHB (n=4, results expressed as means ± SEM.) over time after treatment;

Figure 2 shows pancreatic weight as a percentage of body weight in animals after a single subcutaneous injection of saline or CHB (n=4, results expressed as means ± SEM.) over time after treatment;

Figure 3 shows pancreatic morphology after a single subcutaneous injection of saline of CHB. All H and E stained. **(A)** 48 hours after saline. There is wide separation of ducts (arrows) and islets (I) by closely-packed acinar cells (x500). **(B)** 12 hours after CHB. Note numerous apoptotic acinar cells with characteristic nuclear morphology (arrow) (x1200). **(C)** 18 hours after CHB. Most acinar cells contain pyknotic nuclear remnants (arrowheads) and show cytoplasmic swelling and vacuolation; a few appear normal (arrows) (x900). **(D)** 48 hours after CHB. Advanced secondary necrosis affecting all acinar cells in field. Intact duct is indicated by arrow (x360). **(E)** 96 hours after CHB. No acinar cells remain. Atrophic lobules comprise crowded ducts in a connective tissue stroma. (x200). **(F)** 28 days after CHB. Sparse regenerative acini are seen adjacent to islets (arrows). Note few ducts in a collagenous stroma and prominent fatty infiltration, (x200);

Figure 4 shows pancreatic immunohistochemistry after a single subcutaneous injection of saline or CHB. All with haematoxylin counterstain. **(A)** 24 hours after saline. Widely-spaced keratin-positive ducts (arrows) are separated by closely-

packed keratin negative acinar cells (x160) **(B)** 48 hours after CHB. Widely-spaced keratin-positive ducts (arrows) separated by keratin-negative nonviable acinar cells (x180). **(C)** 96 hours after CHB. Lobules comprise crowded keratin-positive ducts separated by loose connective tissue. No acinar cells are seen, (x180). **(D)** 96 hours after CHB. Ducts are negative for amylase. Note isolated amylase positive epithelial cell (arrow) and perinsular amylase positivity (x160);

Figure 5 shows pancreatic ultrastructure after a single subcutaneous injection of CHB. **(A)** 12 hours after CHB. Adjacent apoptotic acinar cells show well-demarcated crescentic clumped chromatin, large nucleolar remnants (Arrowhead) and whorling of endoplasmic reticulin (arrows) (x3000). **(B)** 18 hours after CHB. Apoptotic acinar cells show nuclear fragments with crescentic clumped chromatin but dilation of endoplasmic reticulin, swollen mitochondria (arrowheads) and plasma membrane rupture (arrow). Contrast with adjacent viable acinar cells (x2800). **(C)** 48 hours after CHB. Note viable duct epithelial cells (D), residual acinar cell cytoplasmic debris (A) and intra-acinar macrophage (M) laden with residual bodies. The pale cell in the duct epithelium (arrow) is also likely to represent an intraepithelial macrophage (x350). **(D)** 96 hours after CHB. Duct with typical indented nuclei and sparse organelles of lining cells. Note mitotic lining cell (M), intraepithelial apoptotic body (arrow), and adjacent collapsed redundant basement membrane (arrowhead) (x2500). **(E)** 96 hours after CHB. Activated and mitotic interstitial fibroblasts. (x.3300). **(F)** 48 hours after CHB. Capillary with intraluminal apoptotic bodies of presumed endothelial cell origin (arrow). Note adjacent intraacinar macrophage (M) with residual body-laden cytoplasm (x.7000);

Figure 6 shows **(A)**. Untreated AR42J acinar cell carcinoma in an athymic rat, showing broad sheets of cells with an area of haemorrhagic necrosis (arrow) (x100).

(B) Untreated AR42J acinar cell carcinoma, (x400), showing multiple mitoses. **(C)** AR42J acinar cell carcinoma 24 hours after a single subcutaneous dose of 140mg/kg CHB, showing widespread apoptosis and an area of surviving cells (arrow). (x100). **(D)** AR42J acinar cell carcinoma 24 hours after a single subcutaneous dose of 140mg/kg CHB, showing widespread apoptotic change (x400).

Figure 7 shows **(A)** Untreated AR42J acinar cell carcinoma, showing primitive acini formation (x1000). **(B)** AR42J acinar cell carcinoma 24 hours after a single subcutaneous dose of 140mg/kg CHB, showing pyknotic and fragmented nuclei and secondary necrosis of cytoplasm (x1000). **(C)** Pancreas of a normal Wistar rat 24 hours after 140mg/kg subcutaneous CHB showing almost total acinar cell death via apoptosis with secondary necrosis (x400). **(D)** Pancreas of athymic rat 24 hours after 140mg/kg subcutaneous CHB showing regional effect with necrotic areas adjacent to well-preserved areas with pyknotic nuclei (x400).

Fig.8 shows an electronmicrograph of apoptotic cells showing condensed marginated chromatin.

Experimental

1. **Determination of Acinar Cell Apoptosis and Regeneration of Acinar Cells with Subcutaneous Administration of CHB.**

1.1 Material and Methods

Synthetic CHB made according to the method of Das and Torssell (Das and Torssell 1983 Tetrahedron 39:2243-2247).

Male Wistar rats weighing 200 – 250 g were caged in pairs and given food and water ad libitum with a 12 hours light-dark cycle. Twelve groups of 10 rats were

divided randomly into 6 test animals and 4 control animals. At time 0, test animals were given 150mg/kg of CHB mixed in 0.5ml sterile normal saline and controls were given 0.5ml sterile normal saline subcutaneously.

For light microscopy, 4 experimental and 4 control animals were killed at 2, 4, 6, 12, 24, 48, 72 and 96 hours and 7, 10, 18 and 28 days using 60 mg intraperitoneal pentobarbitone. Animals were weighed and the pancreas removed, weighed and processed using routine methods. Additional pairs of experimental animals were killed at 18 and 60 hours for electron microscopy and morphological study. Weights were recorded as means ± standard error of the mean (SEM). Differences between means were analysed using Student's t-test.

For quantification of apoptosis, apoptotic cells and bodies, identified using the morphological criteria (Kerr et al. 1995 Method Cell Biol. 46:1-27) and were counted in ten high-power graticule fields (HPF), selected at random, in a histological slide from each animal at 2, 4, 6 and 12 hours with the proviso that mostly acinar tissue filled the field. A group of tightly clustered apoptotic bodies, presumably derived from a single cell, was recorded as a single count. An estimate of the total number of acinar cells per HPF in each slide was made for calculation of an apoptotic index (apoptotic count as a percentage of total acinar cells present). Counts/HPF and apoptotic indices were recorded as means ± SEM for each group. Differences between means were analysed using Student's t-test. Terminal d-UTP nick-end labelling (TUNEL) was not used because it is our experience and the experience of others that it is not always specified for apoptosis, and ultimately, apoptosis must be confirmed morphologically (Ansari et al. 1993 J. Pathol. 170:1-8; Grasl-Kraupp et al. 1995 Hepatology 21:1465-1468).

Immunohistochemistry for cytokeratin an amylase was performed to identify cells in sections as duct (Schussler et al. 1992 Am. J. Pathol 140:559-568; Bouwens et al. 1995 J Histochem Cytochem. 43:245-253) or acinar (Bendayan 1984 Histochem J. 16:85-108) respectively. For cytokeratin, deparaffinized sections were pretreated with 0.1% trypsin, then 0.3% hydrogen peroxide in methanol followed by mouse monoclonal AE1/AE3 anti-cytokeratin at a dilution of 1/40. Secondary antibody was rat anti-mouse biotinylated IgG used at a dilution of 1/400. Antibody-binding was demonstrated using the peroxidase-labelled streptavidin biotin complex method and reactions were developed with 3,3'-diaminobenzidine tetrahydrochloride solution. For amylase, deparaffinized sections were boiled in Target Retrieval Solution then placed in 0.3% hydrogen peroxide in methanol. Primary antibody was anti-rabbit immunoglobulin used at a dilution of 1/500 and secondary antibody was anti-rabbit goat biotinylated IgG used at a dilution of 1/400. Antigen-binding was demonstrated using the peroxidase-streptavidin method developed with Vector VIP peroxidase substrate. All sections were lightly counterstained with hematoxylin.

For electron microscopy, two rats from each test group were deeply anaesthetised with intraperitoneal sodium pentobarbitone. A catheter was inserted into the abdominal aorta and the vasculature flushed in sequence with 1) heparinized normal saline, 2) 1% paraformaldehyde and 1.2% glutaraldehyde in cacodylate buffer and 3) 4% paraformaldehyde and 5% glutaraldehyde in cacodylate buffer (Karnovsky 1965 J, Cell Biol.27:137A-138A). Pancreas was removed immediately, diced and immersed in perfusate no. 3 for two hours, then stored in cacodylate buffer. The tissue was postfixed in 1% osmium tetroxide, stained en bloc in 5% aqueous uranyl acetate, dehydrated through a series of graded alcohols, cleared in propylene oxide, and embedded in an epon-araldite mixture. Semithin sections (1μm) were cut on an

LKB Ultratome V and stained with toluidine blue for viewing. Ultrathin sections from selected areas were picked up on uncoated copper grids, stained with lead citrate and examined with a JEOL-1200 EX11 electron microscope.

1.2 Results

1.2.1 General Observations

Control rats showed no behavioural change and normal weight gain reaching 180% at 28-days (Figure 1). Experimental rats showed discomfort 30 minutes after injection, lost curiosity and became reluctant to move. Body weight fell over the first week and thereafter remained unchanged (Figure 1).

At autopsy, control rats had normal viscera and a pancreatic weight which was constant as a proportion of body weight (Figure 2). Experimental animals showed pancreatic edema from 2 hours, actual pancreatic weight reaching 4.41±0.71 g at 6 hours (compared to 0.78±0.14g in controls, $P<0.001$), then falling. Atrophy was apparent at 7 days and persisted, actual pancreatic weight falling to 0.44 ±0.04g at this time compared to 1.80±0.08g in controls, $P<0.001$.

Changes in pancreatic weight as a percentage of body weight are shown in Figure 2. At 18 and 28 days CHB-treated rats had muscle wasting, abdominal distension and dilated bowel containing undigested food.

1.2.2 Light Microscopy

Control animals showed histologically normal pancreas (Figure 3A). From 2 hours test animals showed mild dilation of acinar lumens and acinar cell vacuolation and depletion of zymogen granules.

Apoptotic acinar cells, evident at 6 hours, showed sharply-defined crescents of clumped chromatin against the nuclear envelope but infrequent fragmentation. Their number reached 178 ±10/HPF at 12 hours (compared to 0.85 ±0.13/HPF in controls, $P<0.001$) or 23.6±7.43% of acinar cells (compared to 0.001% in controls) (Figure 3 B). By 18 hours most acinar cells had chromatin changes of apoptosis but swollen vacuolated cytoplasm indicative of "secondary necrosis" (Figure 3C) which subsequently progressed (Figure 3D). By 96 hours no acinar cells remained (Figure 3E). A few regenerative acini appeared by 18 days, particularly adjacent to islets of Langerhans, but thereafter they did not increase appreciably in number (Figure 3F).

Intercalated ducts were mildly dilated at 4 hours, duct cell mitoses were prominent at 48 hours, and at 96 hours, lobules comprised groups of ducts within a connective tissue stroma (Figure 3E). Small numbers of apoptotic bodies continued to be seen within duct lumens and epithelium. By 7 days ducts had larger lumens and flattened lining epithelial cells. Thereafter the number of ducts decreased with few remaining at 18 and 28 days (Figure 3F).

Interlobular edema was present from 2 hours and interlobular edema from 4 hours; both persisted for 72 hours. The interstitial spaces were acellular before small numbers of mononuclear phagocytes appeared about vessels at 4 hours and within acini at 24 hours. They reached moderate numbers at 48 hours, peaked at 72 hours, then declined markedly by 7 days. Sparse neutrophils were present from 12 hours and mitotic mononuclear phagocytes at 48 hours.

Enlarged mitotically active fibroblasts were seen 48 hours, by 96 hours fibroblasts and collagen enveloped lobules and at 7 days fibroblasts were less prominent and collagen was found both in and around lobules. At 28 days the pancreas comprised largely fat, collagen and islets (Figure 3F).

Islets were not studied in detail. Given the degree of atrophy, however, less islet tissue was apparent than might be expected from simple condensation.

1.2.3 Immunohistochemistry

In controls ducts were positive for cytokeratin and acinar cells negative (figure 4A). At 48 hours in test animals, when few viable acinar cells remained, cytokeratin marked dispersed intact ducts and duct cells (Figure 4B). At 96 hours ducts of atrophic lobules, the only remaining epithelium, were positive for cytokeratin (Figure 4C). Amylase was demonstrated in apoptotic cells at 18 hours, confirming their acinar cell origin. There were no or rare amylase-containing cells at 72 and 96 hours (Figure 4D) with occasional apoptotic bodies staining for amylase. The periphery of islets also showed amylase staining at 96 hours (Figure 4D).

1.2.4 Electron Microscopy

Controls showed normal pancreatic ultrastructure (Ekholm et al. 1962 J. Ultrastruct. Res. 7:61-72; Ekholm et al. 1962 7:73-83). In test animals acinar cell apoptosis was slightly increased at 6 hours and markedly increased at 12 hours, when large numbers of adjacent cells were often affected (Figure 5A). Apoptotic cells showed sharply-defined crescents of chromatin abutting the nuclear envelope, prominent nuclear remnants, whorling of endoplasmic reticulum and structural preservation of organelles (figure 5A) but cellular fragmentation to form apoptotic bodies was uncommon. At 18 hours, apoptotic cells, identified by their nuclear characteristics, remained in situ, but showed dilation of endoplasmic reticulum and nuclear envelopes, swelling and rupture of mitochondria and rupture of plasma membranes (Figure 5 B), so-called "secondary necrosis". This process progressed

such that, by 48 hours, acinar cells were reduced to degraded cellular material associated with small number of intraepithelial macrophages containing ingested apoptotic bodies, degraded cellular material in phagosomes of residual bodies (Figure 5C). By 96 hours, acinar cell debris had been removed (Figure 5D). Ducts and duct cells survived, showing increased mitotic activity, particularly at 60 and 72 hours (Figures 5C and D). Small numbers of ductal intraepithelial apoptotic bodies and surrounding collapsed basement membrane were identified (Figure 5 D).

From 48 to 96 hours, prominent activated and mitotic fibroblasts were seen (Figure 5E). At first collagen was sparse but increased in amount towards 7 days. At 48 hours, mitoses in interstitial macrophages were confirmed and endothelial cell apoptosis was present in interstitial capillaries (Figure 5F); this continued over succeeding days. By 18 days, isolated regenerative acini comprised acinar cells closely resembling acinar cells in control glands.

1.3 Discussion

Within 12 hours of administration of CHB, there is relatively synchronous onset of apoptosis in the majority of acinar cells. This contrasts with the slow onset of apoptosis and gradual increase peaking about the third day that occurs after duct ligation (Walker 1987 Am. J. Pathol. 126:439-451) or the administration of cerulein (Fujimoto et al. 1997 Digestion 58:421-430) or ethionine and a protein-depleted diet (Walker et al. 1993 Pancreas 8:443-449). The sequence is similar but delayed after administration of a copper-depleted diet (Rao et al. 1993. Am. J. Path 142:1952-1957).

The rapid and synchronous onset of apoptosis after CHB administration overwhelms the capacity of duct cells, viable acinar cells and tissue macrophages to rapidly remove apoptotic cells. As a consequence, most of the apoptotic cells remain in situ undergoing progressive swelling, rupture of organelle and plasma membranes and degradative change referred to as "secondary necrosis".

A feature of the CHB model of pancreatic involution is the limited fragmentation of apoptotic acinar cells compared with that seen, for example, after duct ligation (Walker 1987 supra). In the first hours after CHB administration and at lower doses, apoptosis proceeds to cell fragmentation with intraepithelial macrophages at 12 hours engorged with phagocytosed apoptotic bodies making it unlikely that CHB prevents microfilament reorganisation.

Despite early cell death and edema, inflammatory cell infiltration is delayed, reaching moderate density only at 48 hours, the number of neutrophils remaining small throughout. In contrast, cerulein excites a vigorous inflammatory response (Walker et al. 1993 supra; Fujimoto et al 1997 supra).

Acinar cell regeneration is limited to a few acini 10-18 days after CHB administration. After cerulein and ethinione administration, it is rapid and complete once the causative agent is removed (Fitzgerald 1960 Lb. Invest. 9:67-85; Isasser et al. 1986 Pancreas 1:421-429).

2. Determination of Differences in Effect with Oral and Subcutaneous Administration

2.1 Method..

Eight groups of eight male Wistar rats of approximately 200g were divided into 6 test animals and 2 controls. Control animals were given either water orally by gavage or

saline subcutaneously as appropriate to their group. Test animals in each group were given CHB daily for 4 days, with doses for groups 50, 100, 150 and 200mg, orally by gavage in water or subcutaneously in saline. A further group was treated later with 130mg/kg subcutaneously in order to further define the dose-effect. Animals were weighed each day and the day's dose of CHB calculated accordingly. Twenty-four hours after the fourth dose animals were euthanased using 60mg intra-peritoneal pentobarbitone and a full autopsy performed. Organs were removed and weighed before routine processing for histological examination. These were pancreas, brain, thymus, lungs, heart, salivary glands, lumbar vertebrum, paraspinal skeletal muscle, pancreas, liver, intestine, kidney, spleen, seminal vesicle, testicle and prostate.

2.2 Results:

General

Three rats receiving 200mg/kg/day orally died after two doses, the remaining three test and two control animals were euthanased the same day. Three rats receiving 200mglkg/day subcutaneously were dead after one dose, necessitating the euthanasia of the rest of the group. One rat in the 100mg/kg oral test group died after one dose. Overall body weight gain was reduced for all test animals, those animals receiving 130, 1 50 and 200mg/kg/day losing weight daily. Control animals gained 5 grams per day, rats given 50 and 100mg/kg/day gained 1-4 grams per day, rats given 130, 150 and 200 mg/kg/day lost weight. Pancreas and liver weights were not recorded. Spleen, thymus and lung weight was reduced in test animals, more so in high-dose and subcutaneous groups. Kidney weight was unchanged and testicle weight as a proportion of body weight rose.

In the 200mg/kg s/c rats found dead there was haemorrhagic change in liver, bowel, and under claws, pancreatic swelling and erythema, fat necrosis in the abdomen and ascites. One rat in the 150mg/kg oral group had liver infarction and bleeding in the thoracic and abdominal cavities.

Pancreas

The effect of CHB on the pancreas was found to be dependent on both dose and route. As with bodyweight, the subcutaneous route has a stronger effect. There was considerable variability among the rats and within each pancreas.

Oral administration: Rats given 200mg/kg showed edema and moderate apoptosis at 24 hours after their second dose. Other groups were examined 24 hours after the fourth daily dose. The 150mg/kg group showed less effect with mild to moderate apoptosis, and occasional mitosis of acinar cells. With 100mg/kg, there was mild apoptosis of acinar cells but conspicuously increased mitotic activity. The same was true of the 50mg/kg group, with apoptosis less obvious.

Subcutaneous administration: Rats given CHB subcutaneously showed a greater effect on the pancreas for the same dose. In the group given 200mg/kg, the three rats which were dead at 24 hours all had marked Pancreatic edema and moderate apoptosis. The pancreatic lobules were intact. In the three survivors which were euthanased at 24 hours there was apoptosis of approximately 80% of acinar cells with progression to secondary necrosis. In the 150mg/kg group, the effect 24 hours after 4 daily doses was almost complete loss of acinar cells, with lobules composed of ductal complexes, mononuclear inflammatory cells and islets. Both apoptosis and mitosis was present in ductal complexes and perilobular fibrosis was present. In four of the six rats a layer of cells was present around the outer edge of

islets. At 130mg/kg, the same process of acinar cell loss occurred but was less complete, about one-third of acinar cells surviving, and mitosis of acinar cells was seen. Inflammation and mononuclear cell infiltrate were as marked as at the higher doses. Peri-insular ductal cells were also present at this dose. At 100mg/kg, acinar cell loss was about 20 – 80% with greater variability between rats and within pancreases. 50mglkg caused only mild apoptosis but mitosis was increased.

Other Organs

Of other organs examined only the liver showed significant changes. The effect on the liver was greater when CHB was given by the oral route.

Oral CHB: In the three rats that died after 200mg/kg all had widespread necrosis of the liver. Nuclear changes were those of apoptosis with chromatin condensation and fragmentation. Hepatocytes were necrotic and there were patches of haemorrhage. In the three rats that survived until euthanasia, one rat had the same degree of liver damage, another had focal inflammatory foci and loss of hepatocytes, another had fine vacuolation of most hepatocytes with apoptotic bodies in sinusoids.

150mg/kg orally induced liver changes in half the rats with focal loss of hepatocytes, necrotic patches and clusters of sinusoidal cells in portal areas of the lobule. Lower doses had no discernible effect.

Subcutaneous CHB: Four daily doses of 150mg/kg induced changes of liver necrosis in subcapsular areas in contact with pancreas. The remainder of these livers were normal.

2.3 Discussion

The effect of CHB on the pancreatic acinar cell is dose-related. The lesser effect of the same dose by oral route is not surprising, attributable to less complete absorption from the GIT and/or metabolism and neutralization in the liver as a first-pass effect. When 200mg/kg is given orally in water it has a more toxic effect than when in a corn-oil vehicle, causing fatal liver necrosis in half the animals and severe hepatocyte damage in the survivors. Even at 150mg/kg orally, liver damage was significant with only mild-moderate pancreatotoxicity. It is expected that absorption of a water solution would be more complete and rapid than that of a suspension in oil. Liver necrosis was evenly widespread. In the group given 150mg/kg subcutaneously, liver necrosis was confined to subcapsular areas in contact with pancreas, suggesting a local effect possibly due to enzyme action. The effect on the pancreas of this hepatotoxic-dose is moderate. In a study of the pancreatotoxic effects of CHB given in three vehicles (Wallig et al, 1989), the liver was not studied. If CHB is to be used for its pancreatotoxic effects, then the oral route should be avoided.

3. *In Vivo* effect of CHB on Pancreatic Carcinoma

3.1 Method

Athymic rats 200 –250g were purchased from the Animal Resources Centre, Western Australia. Four experiments were performed using slightly different doses with the intention using a dose just sub-lethal in order to assess maximal effect.

Experiment 1: *Ductal* carcinoma cells (2×10^6 cells of Capan 2) were injected into the left flank of 7 nude rats. Sixty days later, tumour nodules were 1 –2cm diameter. By the time tumours were ready for testing, rats weighed approximately 300g. 135mg/kg CHB (absolute dose 38 - 40mg) was given into ventral abdominal subcutaneous tissue in 0.5ml sterile saline with 2 controls getting saline only. At 18

hours, all test rats were dead. Autopsies were performed taking specimens of carcinoma, pancreas and liver for processing.

Experiment 2: 6 rats were inoculated in the right flank with 5×10^6 cells of Ar42J rat *acinar* cell carcinoma. By 13 days all grew tumours 1 – 2cm diameter. CHB at a dose of 125mg/kg mixed in 0.5ml saline (absolute dose 26 – 32.5mg) was injected into ventral abdominal skin of all 6 rats. In this experiment there were no controls. All rats survived until eighteen hours later when they were euthanased using 60mg intraperitoneal pentabarbitone. Carcinoma, pancreas and liver were dissected out and placed in formalin for processing.

Experiment 3: 14 rats were inoculated with 5×10^6 cells of Ar42J *acinar* cell carcinoma. Seven rats were given CHB at 140mg/kg (approximately 30 – 32mg absolute) and another 7 rats were given saline only. At 18 hours all rats had survived and were euthanased, pancreas, liver and carcinoma removed and taken for processing.

3.2 Results

Experiment 1: *Ductal* carcinoma nodules were not effected by CHB. Histological appearance was the same in both test and control groups.

Experiment 2: At lower doses of CHB, 1 of 6 test rats had evidence of a cytotoxic effect with widespread apoptosis and secondary necrosis. Apoptosis was as described previously, with crescentic clumping of chromatin, as well as wheel-rim clumping around the nuclear membrane. Fragmentation of cells was not obvious as

described in other settings of massive synchronous apoptosis. In order to distinguish the effect of CHB from patchy haemorrhagic necrosis, an effect was regarded as present only if little viable tumour remained.

Experiment 3: Control tumour nodules were composed of sheets of uniform large cells with dilated vascular channels and some areas of haemorrhage and necrosis (Figure 6A and B, Figure 7A). Patches of apoptotic cells were present in places, particularly near haemorrhages. Mitotic rate varied but in places was very high. Three of 7 rats had a marked effect (Figure 6C and D, Figure 7B), a further 2 had about half surviving and the remaining 2 rats had no discernible effect.

Three test rats with acinar cell carcinoma that died of CHB toxicity in preliminary dose-testing experiments had almost total cell death in the tumour nodules. The fact that one rat had some patches of surviving tumour cells makes post-mortem change unlikely to be responsible for the apoptotic appearance.

The effect on pancreas was different in athymic and Wistar rats (Figure 7C and D). Athymic rats had a regional effect in the pancreas with areas of secondary necrosis juxtaposed with well-preserved areas of acinar tissue, albeit with pyknotic nuclei.

3.3 Discussion:

There is no discernible effect of CHB on ductal cells in the normal pancreas. It is therefore not surprising that malignant ductal cells are not substantially affected by CHB. Normal acinar cells, however, are sensitive and can be eliminated by a single subcutaneous dose of 140mg/kg in the Wistar rat. Only very limited regeneration of acinar cells was seen at 28 days, and this was in the peri-islet areas.

The usual picture of apoptosis is that nuclear changes are followed rapidly by cytoplasmic condensation, blebbing and fragmentation. As described in other settings of massive synchronous apoptosis fragmentation was not obvious.

In the nude rat tumour nodules the effect of CHB appeared to be total regional apoptosis or nothing. No part effect was seen. At the lower dose, 1 of 6 rats had an almost total cell kill, 5 had no discernible effect. At the higher dose, 3 of 7 had a marked effect with few areas of viable cells, and a further 2 of 7 had apoptotic change in about half the tumour section. In unaffected tumours, there was no increase in apoptosis and no decrease in mitosis. Areas of tumour were either totally apoptotic or seemingly unaffected.

The effect on pancreas in the athymic rat is different from that in the Wistar rat. Despite marked edema as expected, the appearance of the pancreas is unusual in that nuclei in both necrotic and well-preserved areas are pyknotic. Patches of apoptotic cells are seen in the liver. It may be that a thymic humoral component is involved in the widespread apoptotic process in Wistar pancreas, without which the lesion is different.

An advantage of the current invention is that the method of treatment can be used to kill acinar carcinoma cells in the pancreas and spread throughout the body. Acinar carcinoma cells are resistant to radiation treatment and chemotherapy. While it may be possible to surgically remove the pancreas and hence the acinar carcinoma cells in the pancreas, detection of acinar cell carcinoma usually only occurs after the acinar carcinoma cells have spread from the pancreas. Furthermore the treatment only killed the acinar cells, and other cells in the pancreas appeared to be biologically functional after the treatment. Thus a patient would possibly avoid becoming diabetic

with functionally active islet cells in the pancreas. With surgical removal of the pancreas a patient becomes diabetic and insulin must be administered.

It will of course be realised that while the foregoing has been given by way of illustrative example of this invention all such and other modifications and variations thereto as would be apparent to persons skilled in the art are deemed to fall within the broad scope and ambit of this invention as defined in the claims appended hereto.

CLAIMS:

1. A method of eliminating acinar cells in a patient by administration of cyanohydroxybutene in a therapeutic window selected to provide substantially non-regenerative apoptosis of said acinar cells.

2. A method according to claim 1, wherein said therapeutic window is selected to minimise liver damage in said patient.

3. A method according to claim 1 or claim 2, wherein said therapeutic window is selected to eliminate substantially all acinar cells in said patient.

4. A method according to any one of claims 1 to 3, wherein said administration is by means selected from subcutaneous, intraperitoneal, and intramuscular administration, and intravenous infusion.

5. A method according to claim 4, wherein said administration is subcutaneous.

6. A method according to claim 5, wherein said cyanohydroxybutene is presented in an aqueous administration medium.

7. A method according to any one of claims 5 and 6, wherein said cyanohydroxybutene is administered at a dosage within the range of 5-300mg CHB/kg of body weight.

8. A method according to claim 7, wherein said patient is selected on the basis of said acinar cells including acinar carcimoma cells.

9. A method for treating pancreatic disease including administering to a patient a therapeutically effective amount of cyanohydroxybutene to cause substantially non-regenerative apoptosis of acinar cells in the patient.

10. A method of treating pancreatic carcinoma having acinar cells including preparing a cyanohydroxybutene (CHB) formulation; and
administering a one or more sub-lethal doses of the CHB formulation to a subject with acinar cell carcinoma, wherein the treatment causes substantially non-regenerative apoptosis of malignant acinar cells in a patient.

11. A method as claimed in claim 10 wherein the CHB dose is within a range of 5-300mg CHB/kg of body weight.

12. A method as claimed in claim 11 wherein the CHB dose is within the range of 125-160 mg CHB/kg of body weight.

13. A method as claimed in claim 10 wherein the carcinoma includes acinar cell carcinoma and pancreatic carcinoma containing a mixed population of cells including acinar cells.

14. A method as claimed in claim 10 wherein the CHB formulation is administered by subcutaneous injection.

15. A method as claimed in claim 10 wherein CHB molecule is conjugated to a ligand molecule which is able to bind to an acinar cell surface receptor.

16. A method as claimed in claim 10 wherein only one dose of CHB is administered.

17. A method of treating acute or chronic pancreatitis including preparing a cyanohydroxybutene (CHB) formulation; and administering a one or more sub-lethal doses of the CHB formulation to a subject with acute or chronic pancreatitis wherein the dose includes an amount of CHB that causes apoptosis of substantially all acinar cells and substantially no regeneration of acinar cells.

18. A method of treating acute or chronic pancreatitis as claimed in claim 17 wherein the CHB dose is within a range of 5-300mg CHB/kg of body weight.

19. A method of treating acute or chronic pancreatitis as claimed in claim 17 wherein the CHB formulation is administered by subcutaneous injection.

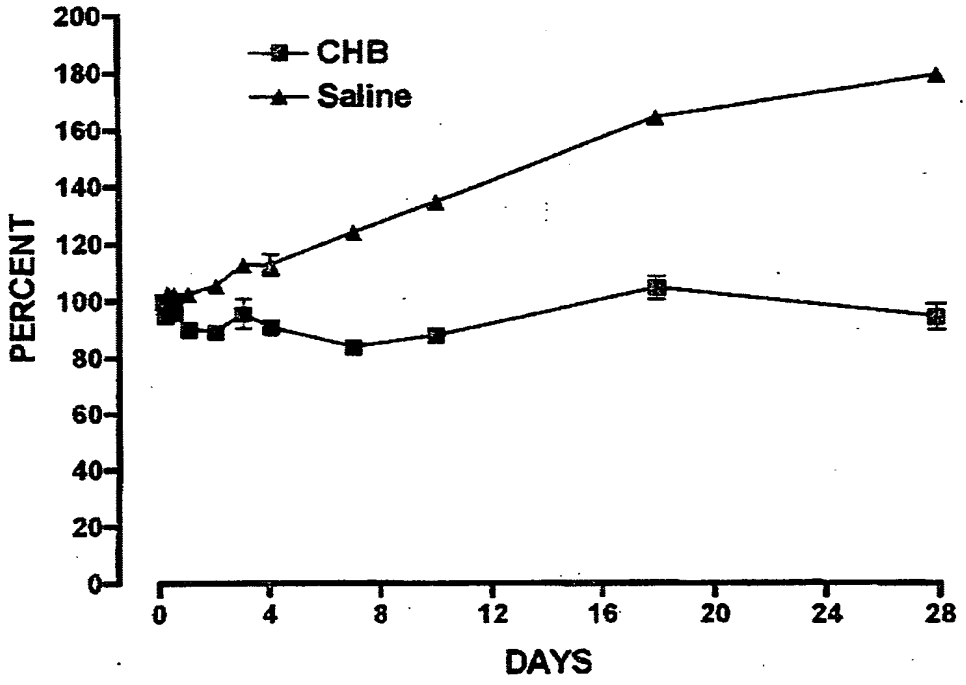
CHB
Saline
PERCENT
200
180
160
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
28
DAYS

Figure 1

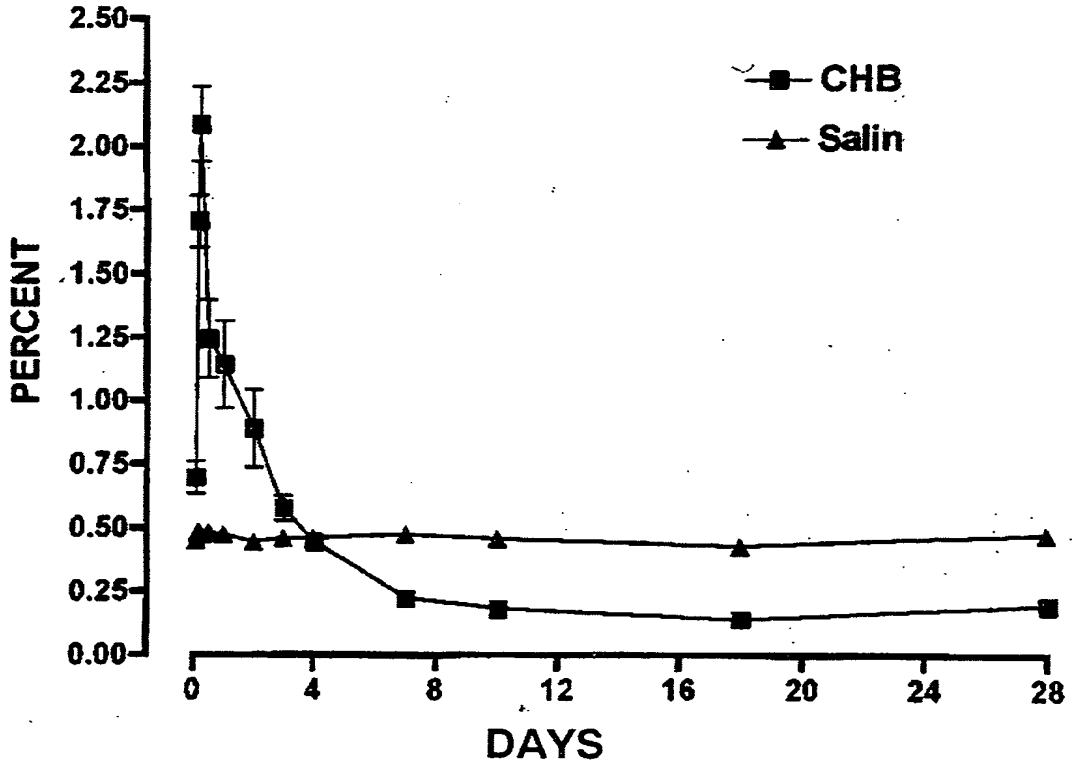
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
PERCENT
CHB
Salin
0
4
8
12
16
20
24
28
DAYS

Figure 2

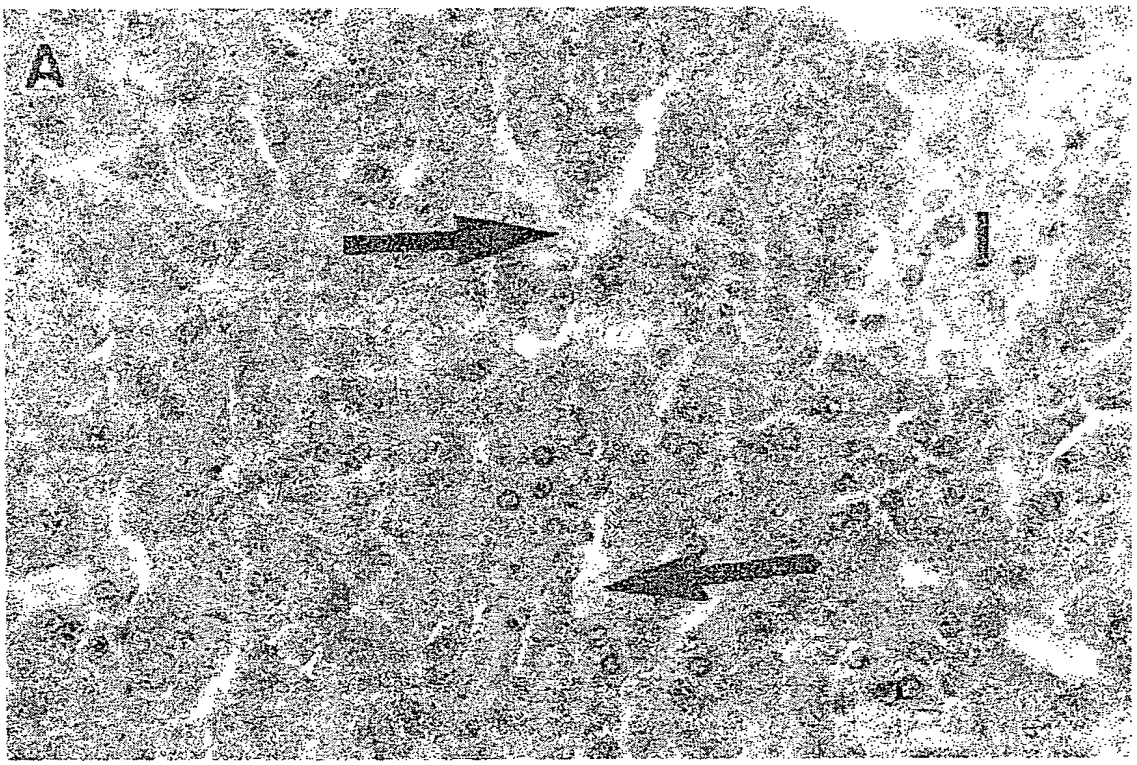

Figure 3A

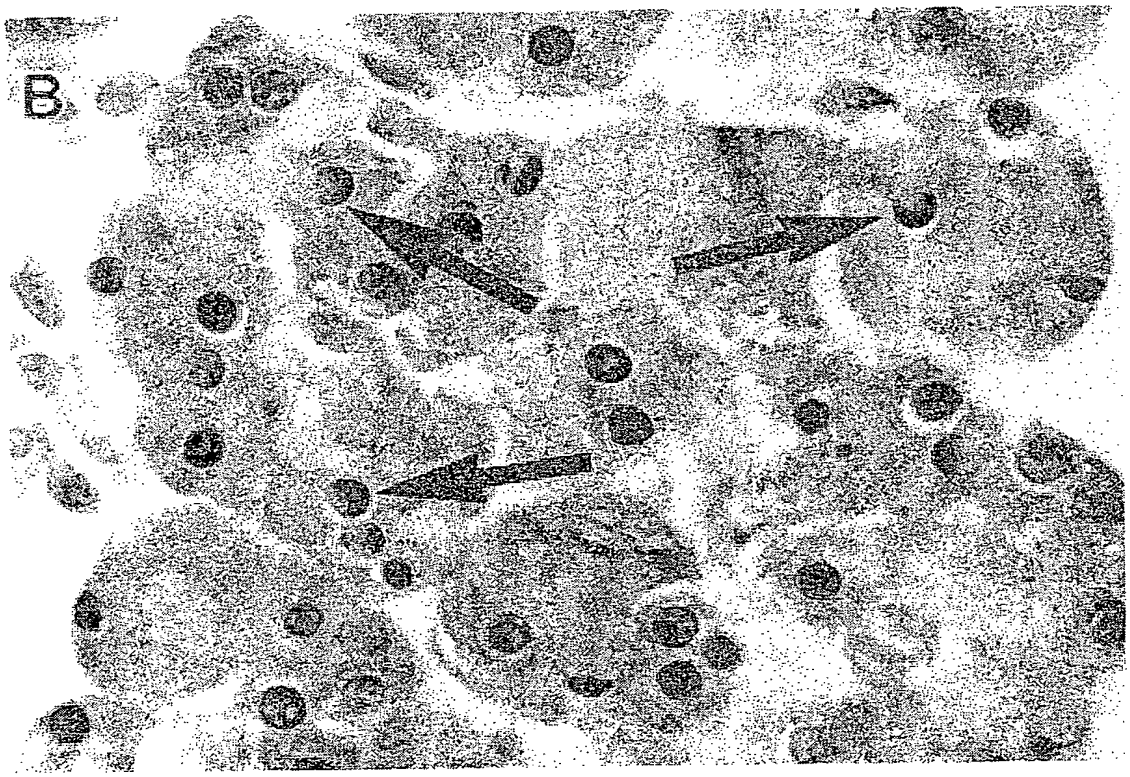

Figure 3B

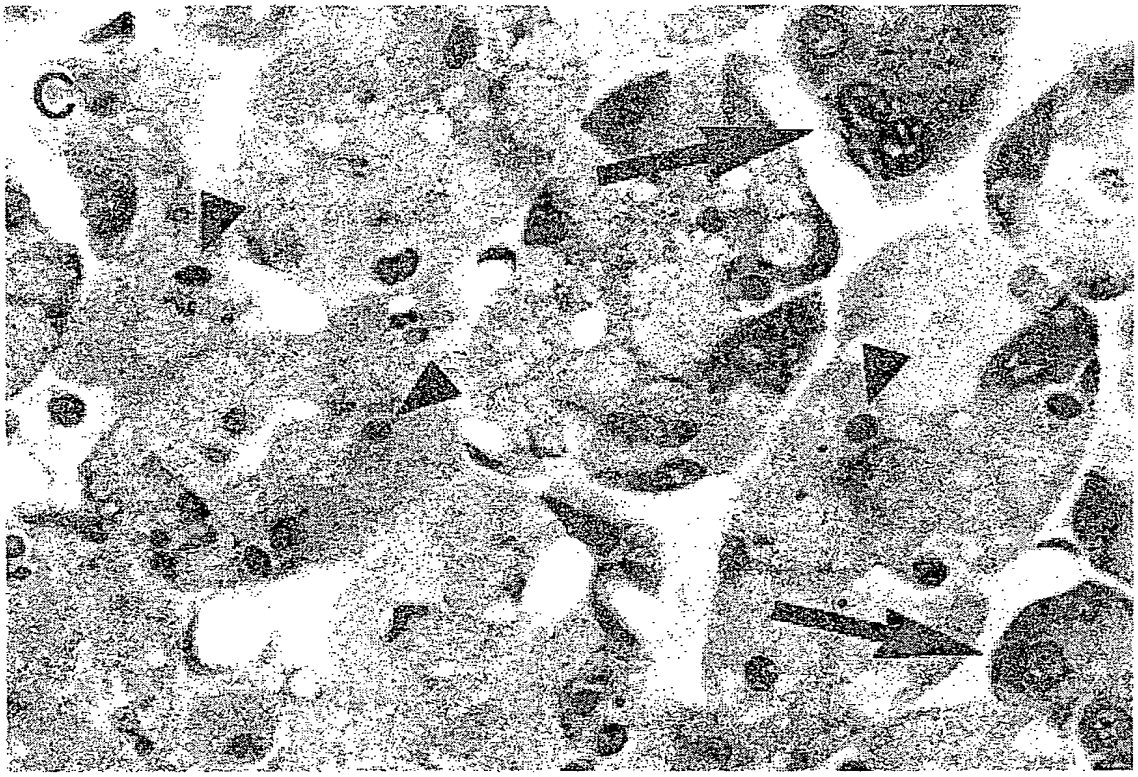

Figure 3C

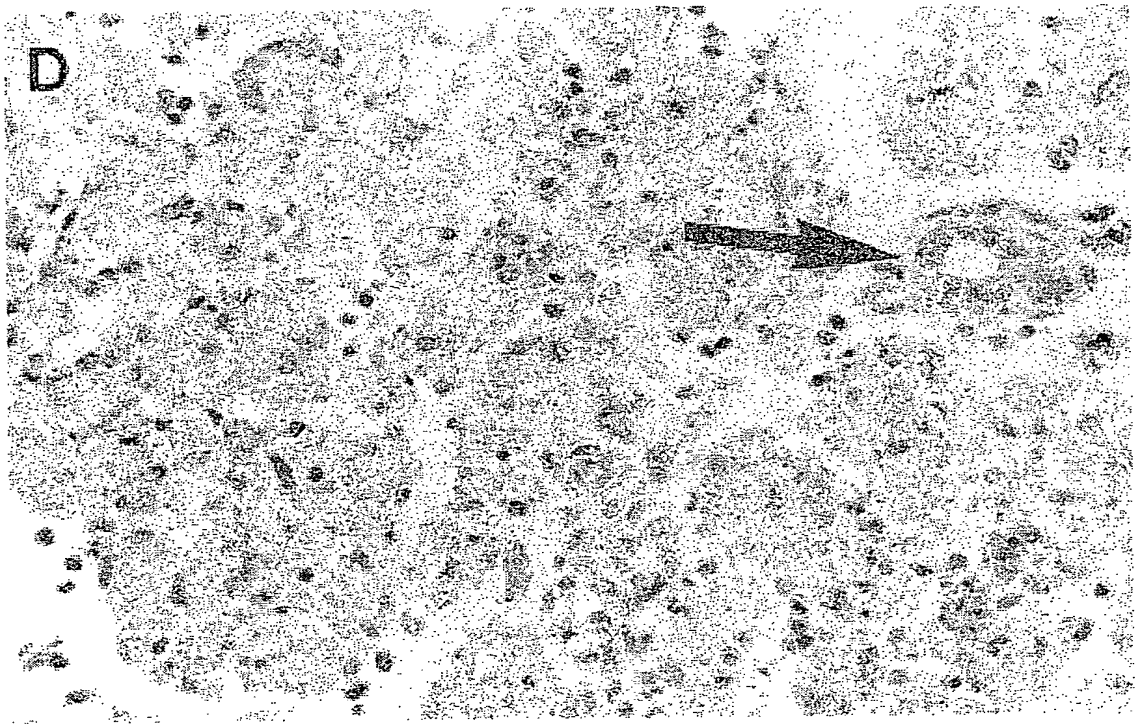

Figure 3D

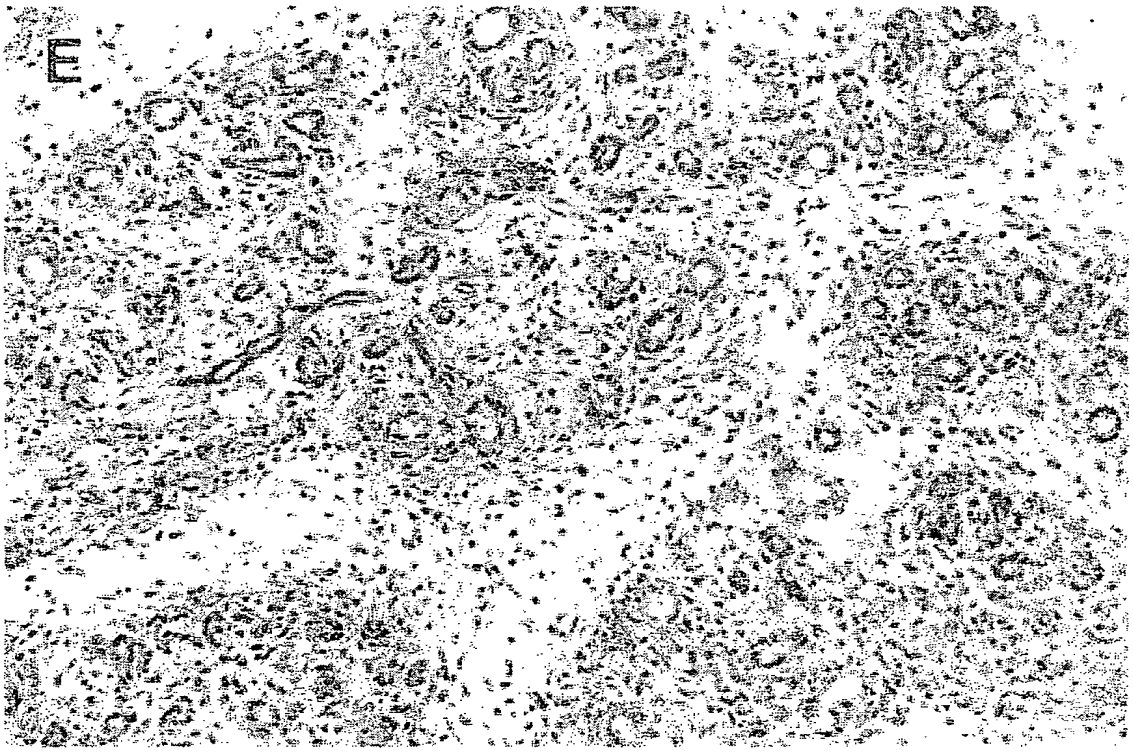

Figure 3E

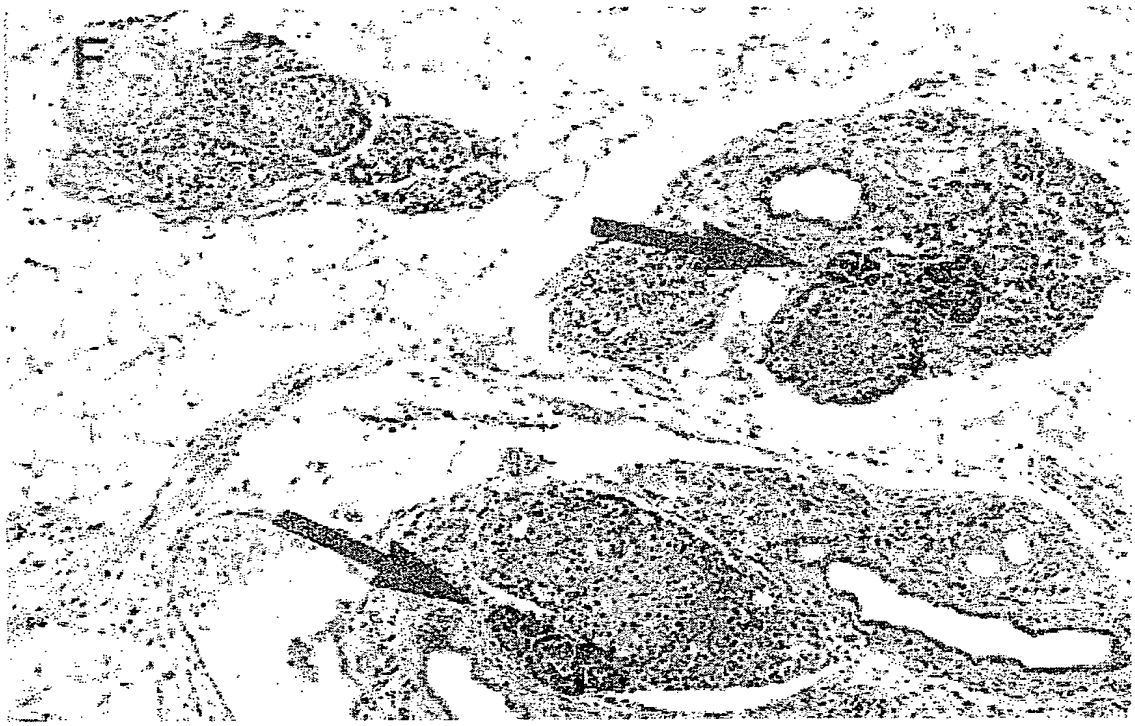

Figure 3F

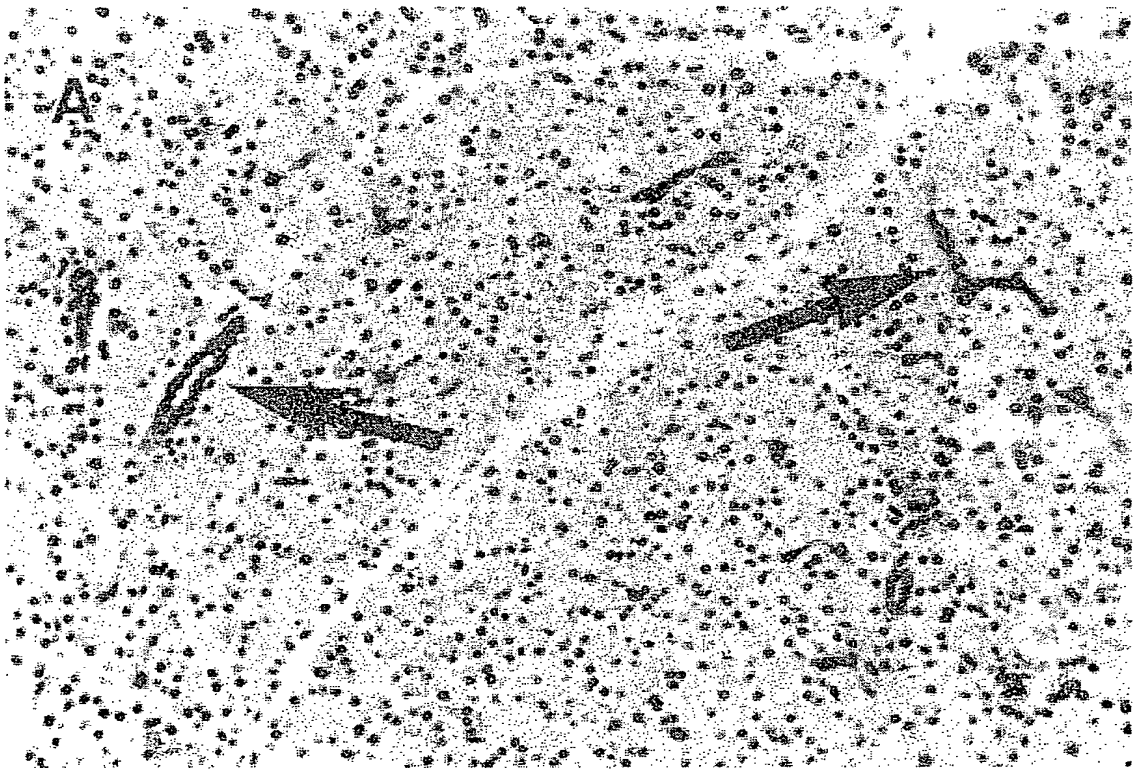

Figure 4A

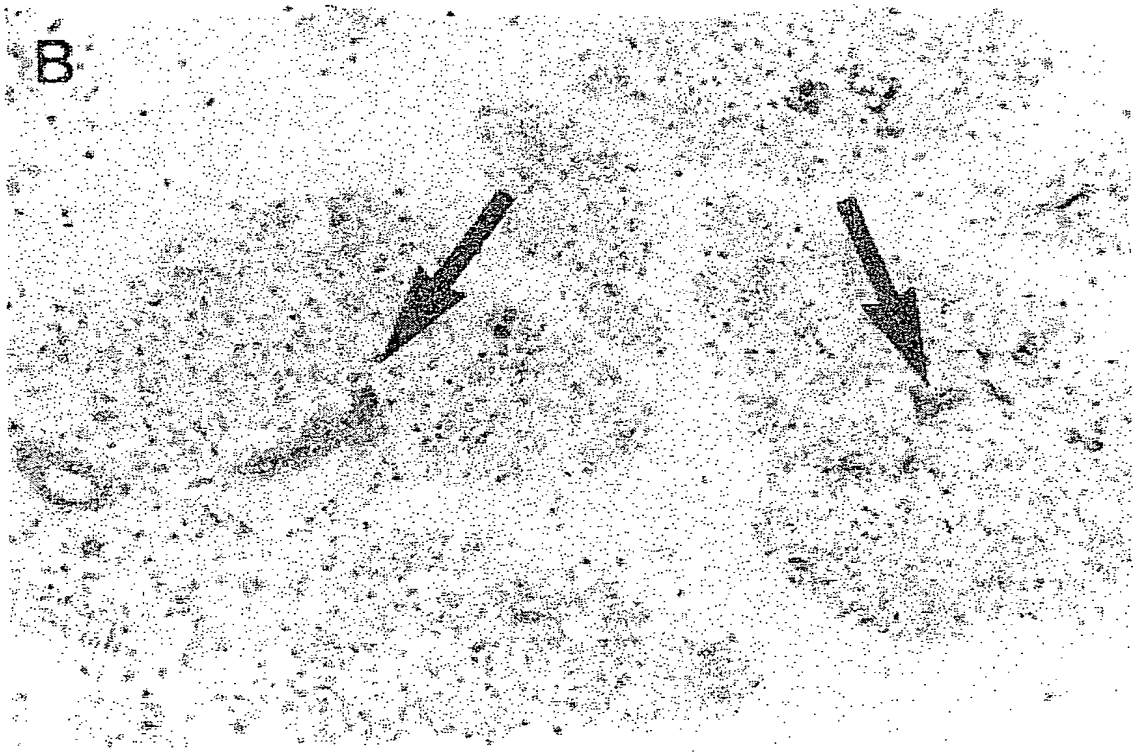

Figure 4B

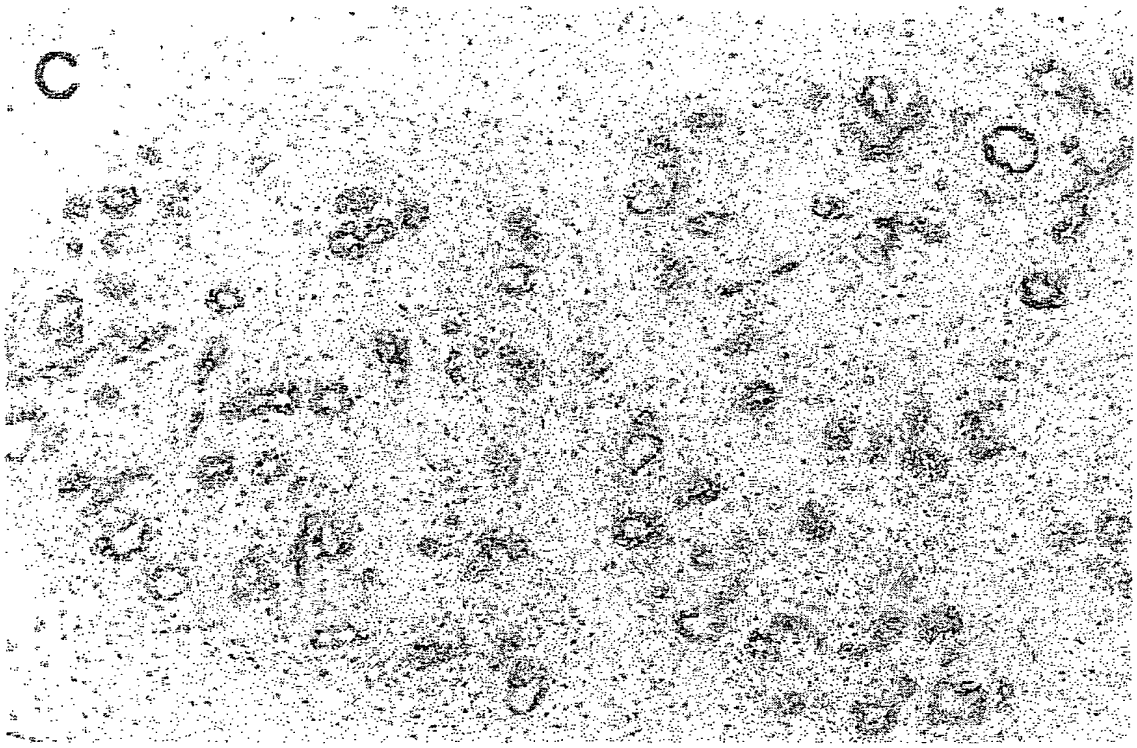

Figure 4C

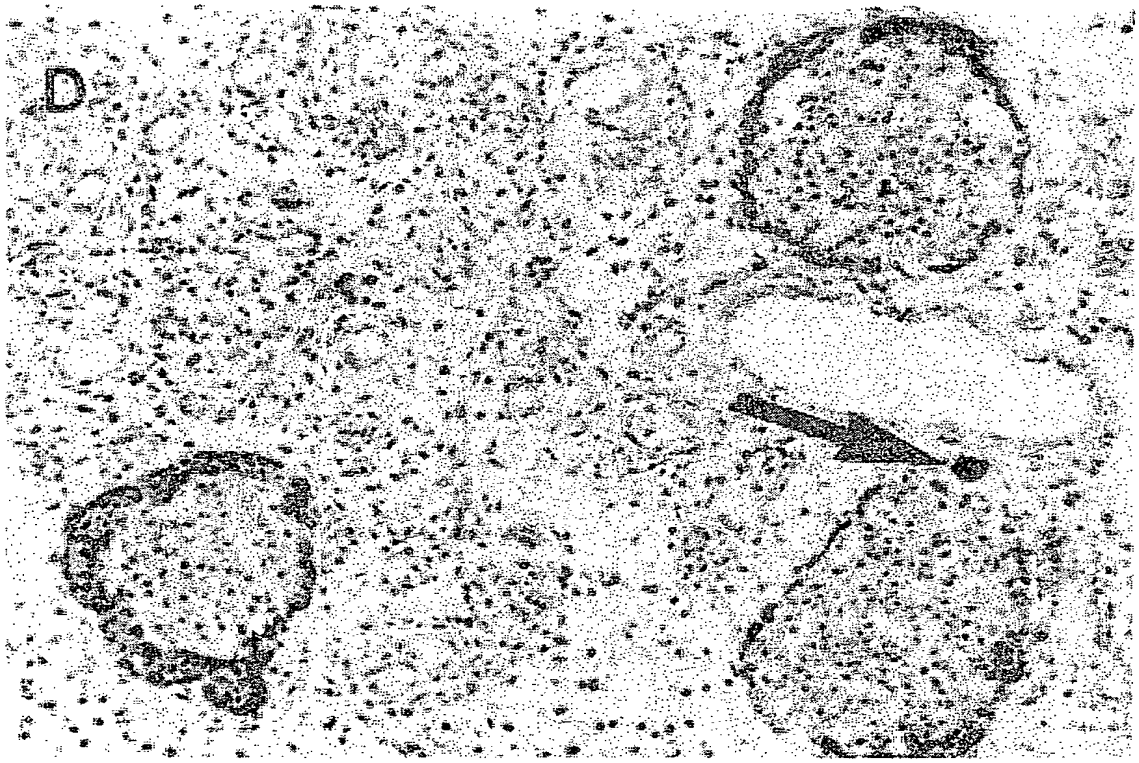

Figure 4D

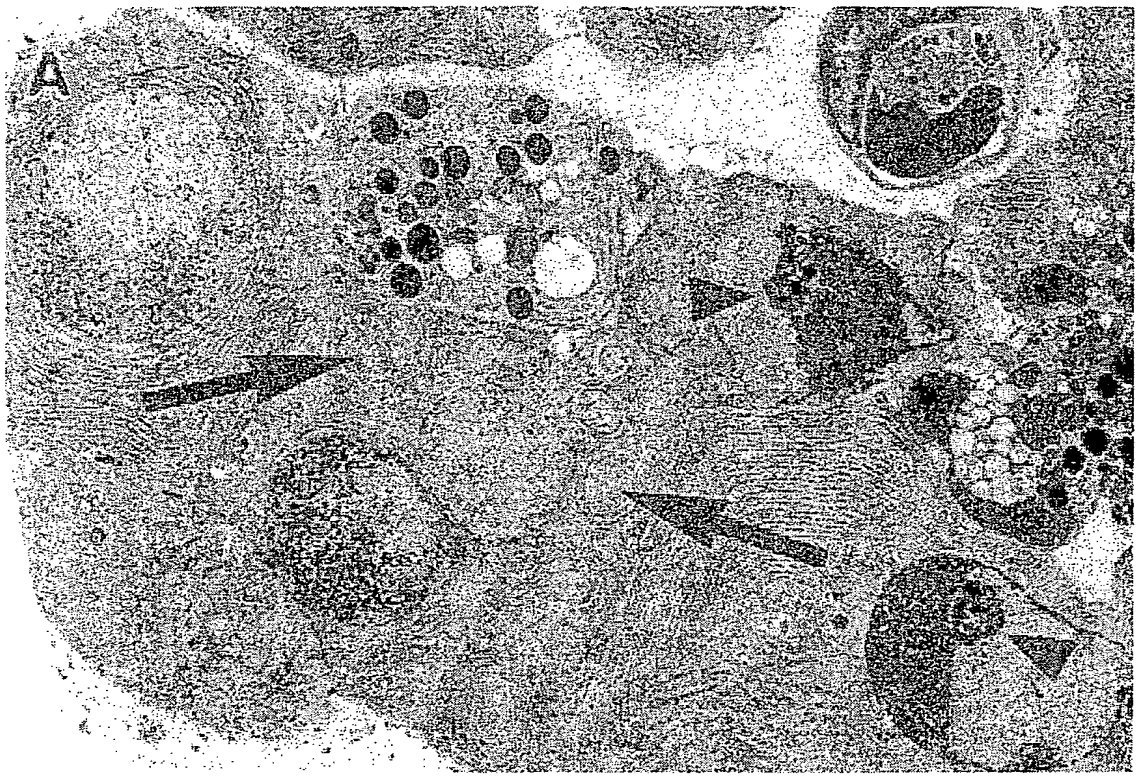

Figure 5A

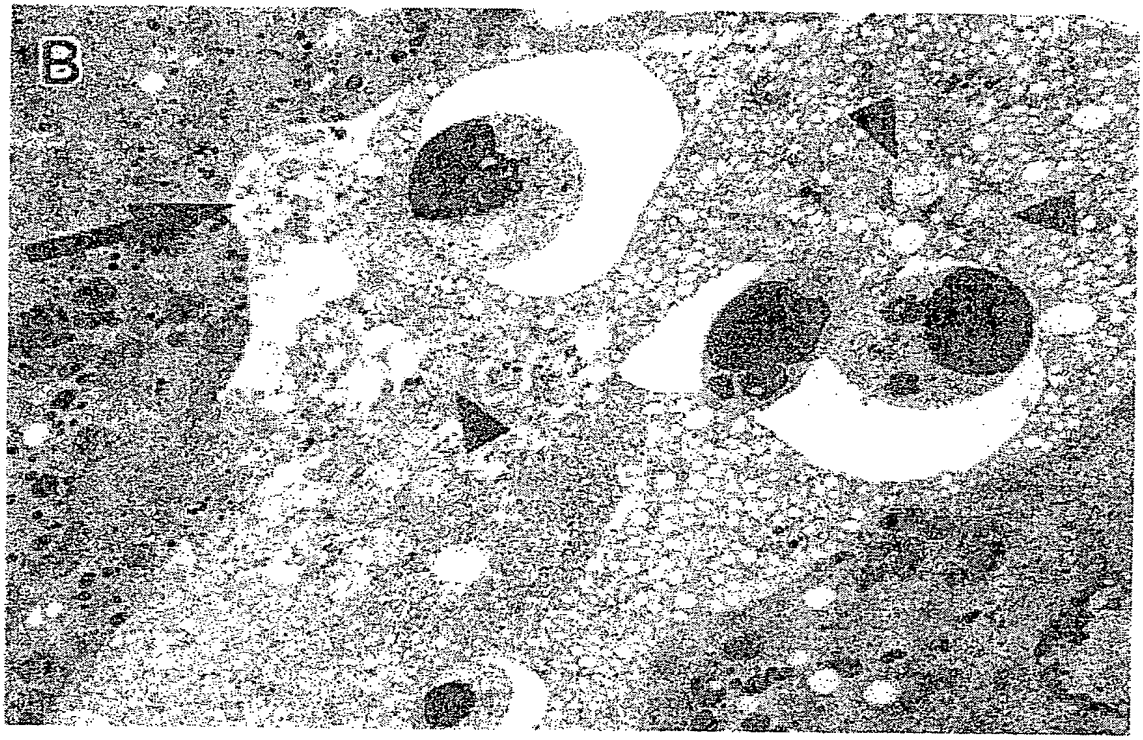

Figure 5B

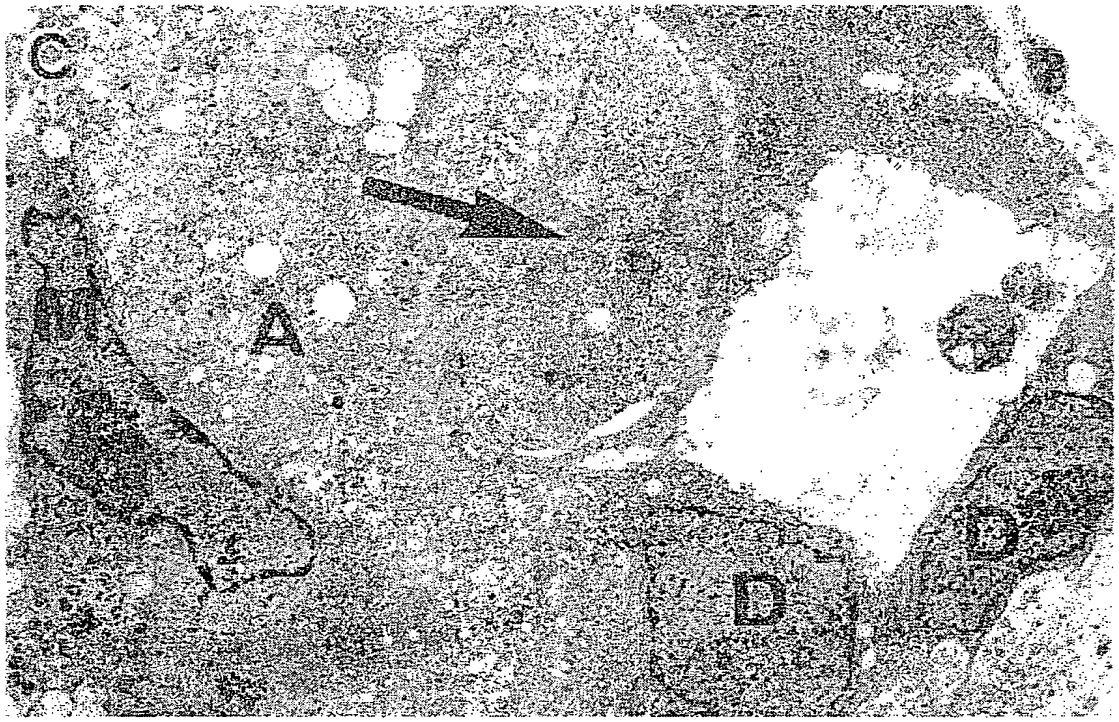

Figure 5C

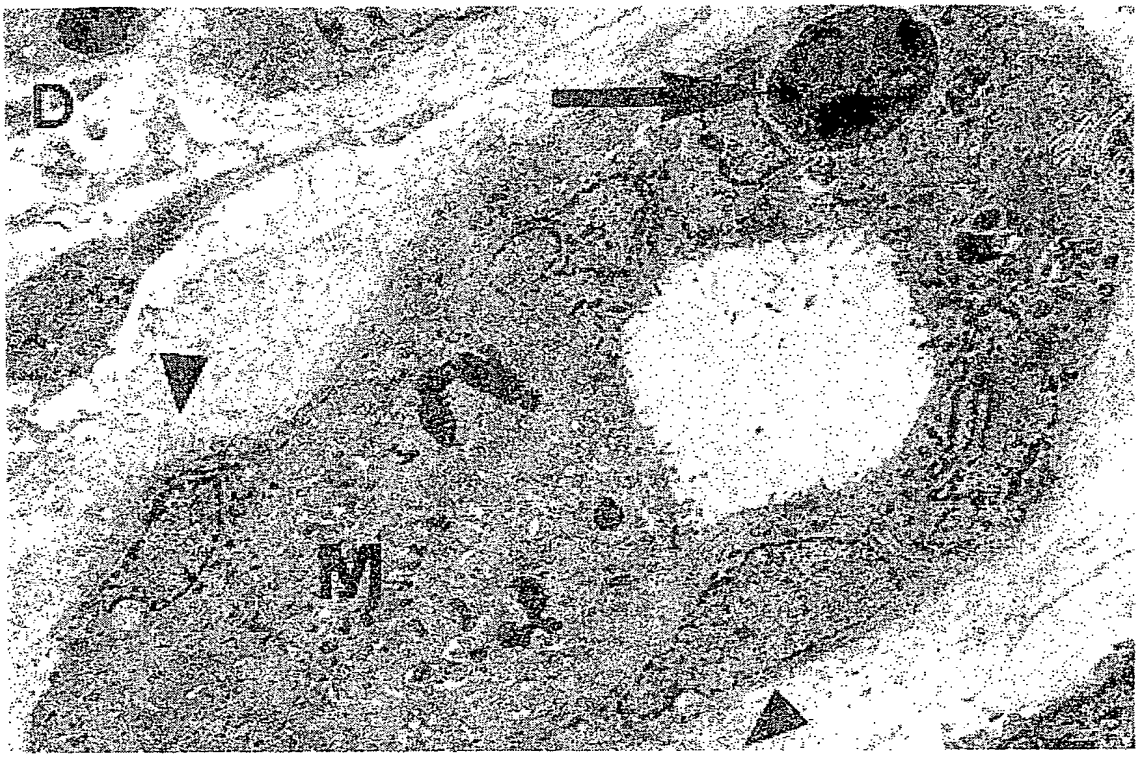

Figure 5D

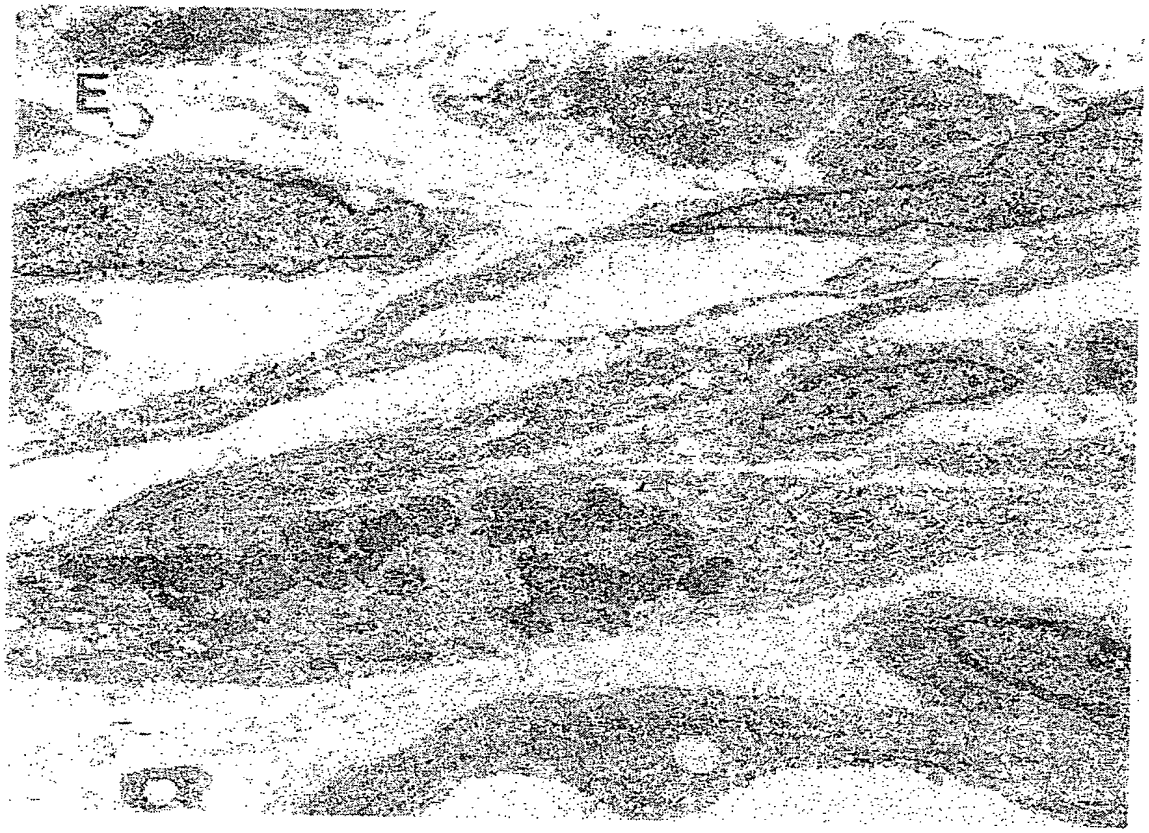

Figure 5E

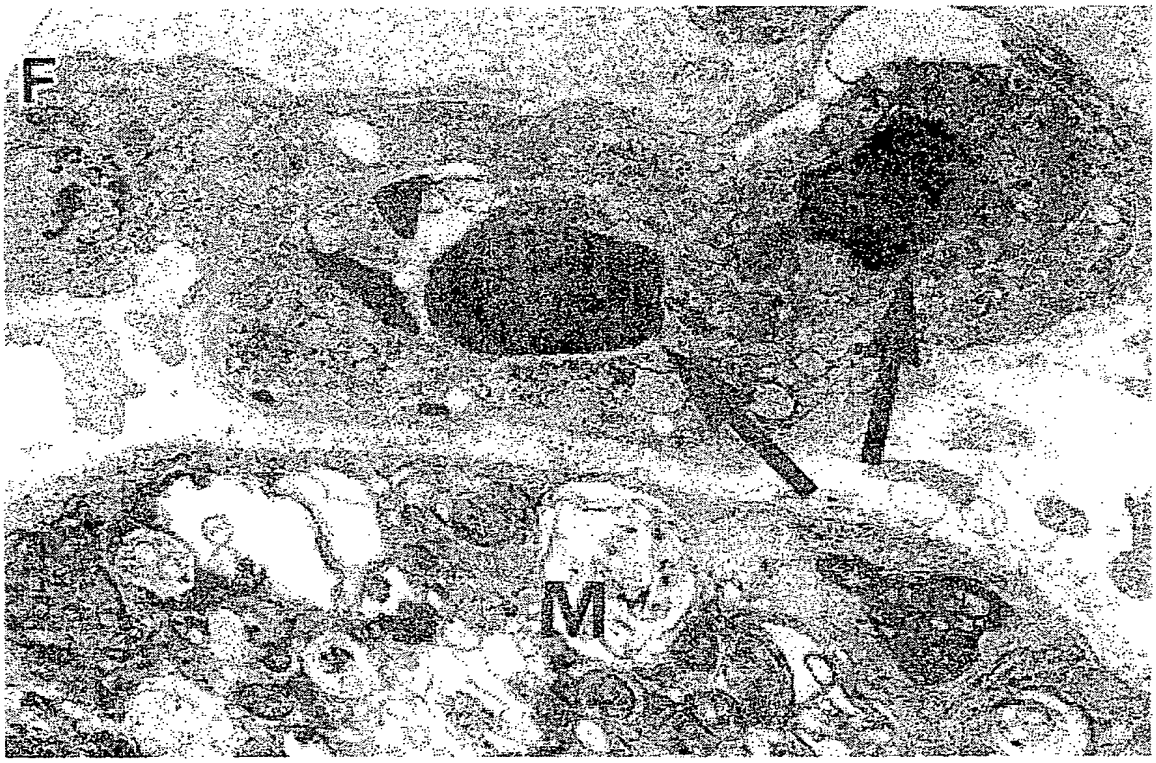

Figure 5F

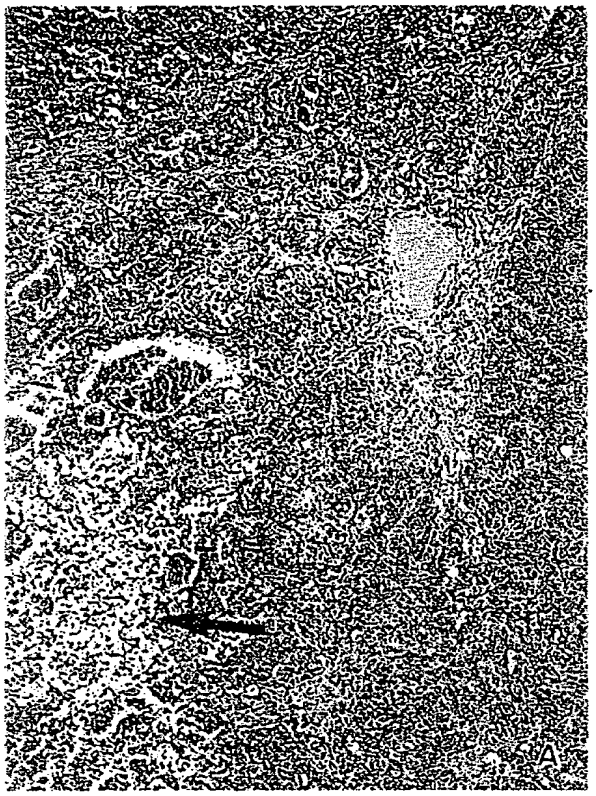

Figure 6A

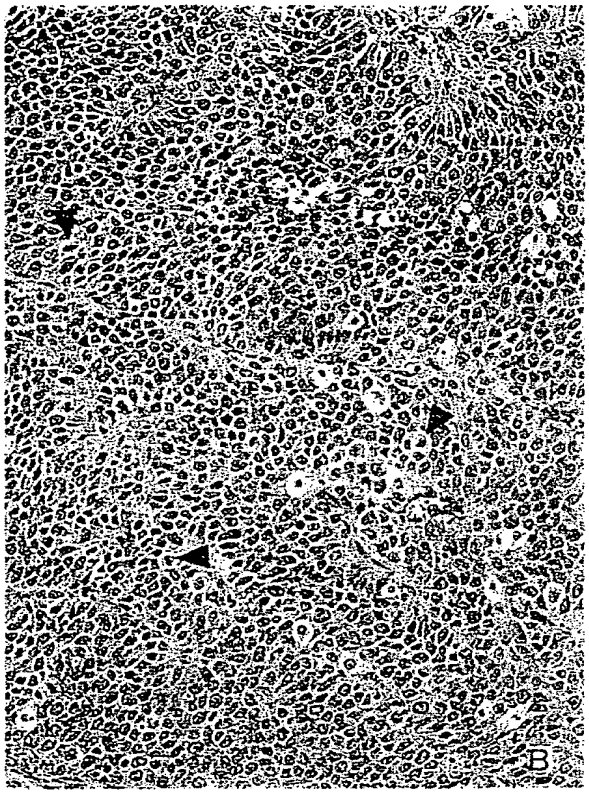

Figure 6B

Figure 6C

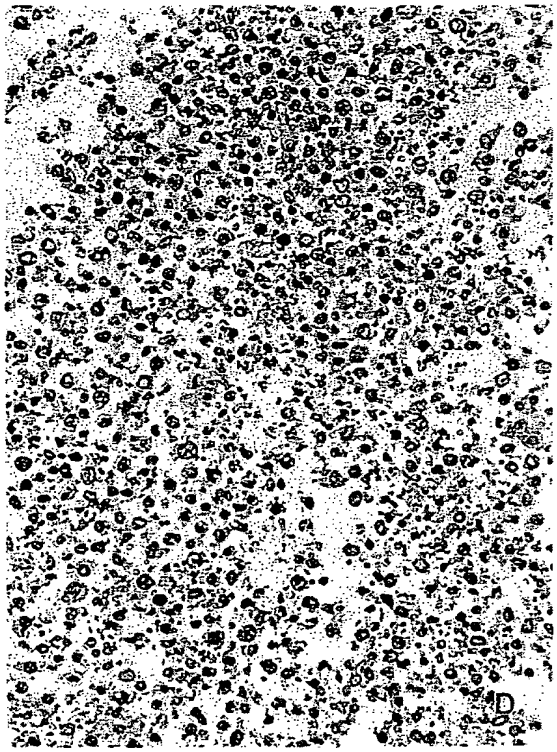

Figure 6D

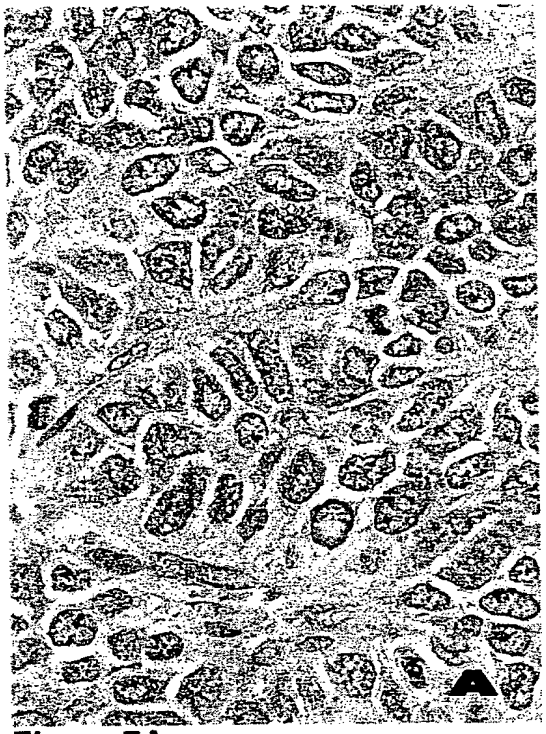

Figure 7A

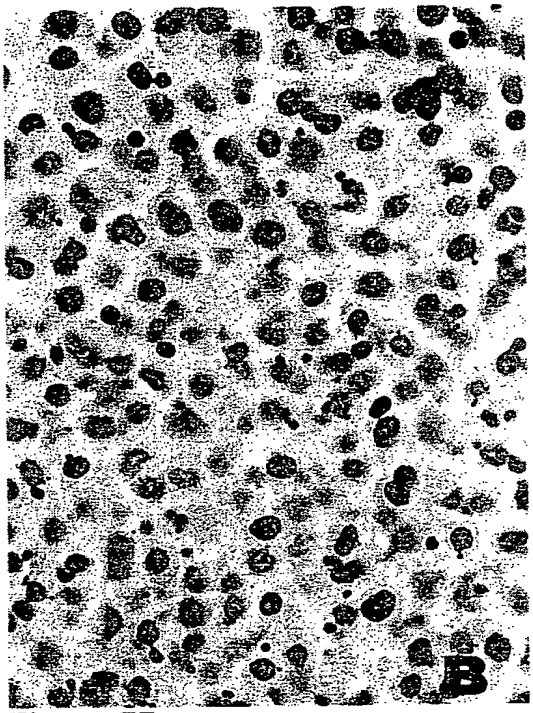

Figure 7B

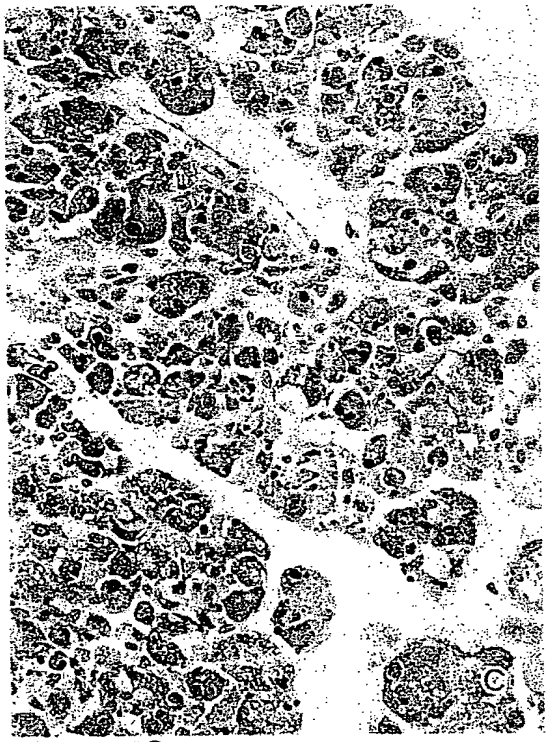

Figure 7C

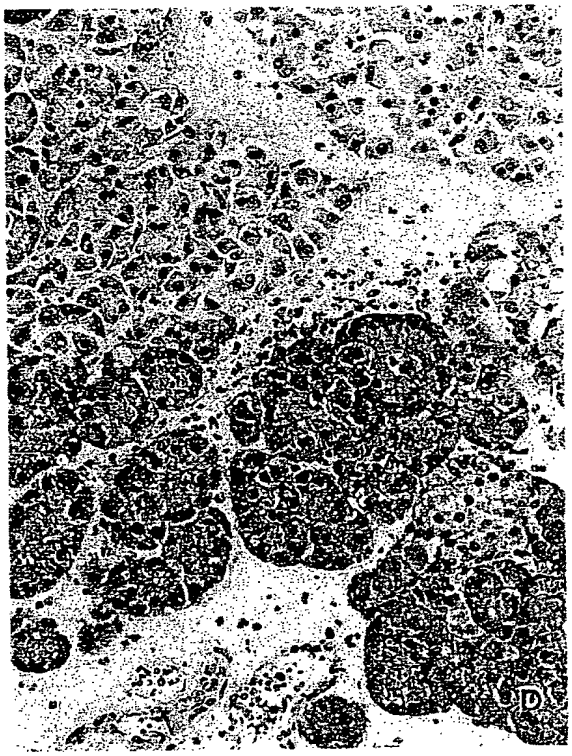

Figure 7D

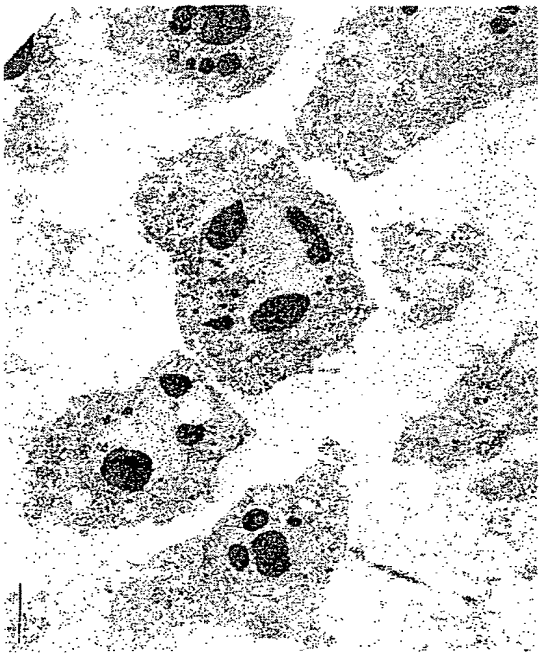

Figure 8

[redacted] [redacted]

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 00/01026

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[7]: A61K 31/275, A61P 1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT } (acinar and carcinoma) or (pancreatitis or acinar) or (pancreat and carcinoma) and cyano () hydroxy() butene or CBH
Medline and CAS} keywords as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pancreas, Vol 6, No. 2, pp 168-174, 1991 Maher, M et al "The Acute Pancreatotoxic Effects of the Plant Nitrile 1-Cyano-2-hydroxy-3-butene." see esp p 173	1-19
X	Food and Chemical Toxicology. Vol 26, No 2, pp 137-147, 1998 Wallig, M et al. "Selective Pancreatotoxicity in the Rat Induced by the Naturally Occuring Plant Nitrile 1-cyano-2-hydroxy-3-butene.", see abstract and discussion p 140	1-19
X	Fundamental and Applied Toxicology. Vol 14, pp 144-159, 1990 Wallig, M et al "Enhancement of Pancreatic and Hepatic Glutathione Levels in Rats during Cyanohydroxy butene intoxication". See pp 144-145	1-19

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2000	-6 NOV 2000

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No.: (02) 6285 3929	TAMARA NIZNIK Telephone No.: (02) 6283 2422

INTERNATION[redacted]CH REPORT

[redacted]nal application No.
PCT/AU 00/01026

C (C ntinuati n). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biochemical and Biophysical Research Communications. Vol. 246, pp 476-483, 1998. Bhatia Madhav et al "Induction of Apoptosis in Pancreatic Acinar Cells Reduces the Severity of Acute Pancreatitis" see abstract and p 476, p480-p483.	1-19